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The Distribution of Alkaline Phosphatase in Normal and Pathologic Human Skin

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I. Introduction

Alkaline phosphatase is an enzyme widely distributed in various tissues of the body. There are only few reports concerning alkaline phosphatase * in skin disease, and the norms for healthy tissue have not been completely established.

It was the purpose of this work to perform adequately controlled investigations in order to elucidate the distribution of AP in normal human skin and in 68 dermatologic conditions.

A. The Physiologic Significance of Alkaline Phosphatase

Although AP is found in all organs of the body, its role in the physiology of organs, other than bone, is more or less obscure.

The physiologic role of AP is best understood in the processes of calcification and ossification.[47,50,59,83,88,100,101,119,140] Alkaline phosphatase is also found histochemically in certain sites of fibroplasia,[6,11,16,30,36,55,70,78,121,142,166,182] inflammatory reaction,[83,162] glandular secretion,[31,37,39,40,48,49,122,147,165,166] absorption and resorption by epithelium, histodifferentiation,[92,93,135,137] and transmembranous solute passage,[138] but its significance in these latter phenomena remains unsolved or, at best, is speculative.

It is probable that AP is in some manner involved in carbohydrate,[9,12,40,55,94,130,191] protein,[6,19,21,25,27,47,138,166,179] and possibly fat metabolism.[57,58,129,138,168]

Alkaline phosphatase activity is regulated in part by the endocrine systems.[6-8,11,13-15,45,46,85,89,90,98,99,105-108,113,115,132,150,160,185]

In skin physiology the role of AP remains for the most part an enigma, but it serves a variety of processes which require the mobilization of phosphate ions or entail dephosphorylation as steps in the metabolism and transport of a large group of phosphate-containing compounds.

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Dr. Arthur Bernard Hyman suggested this study. He has guided me in this work from its conception to its completion, in the reading and interpretation of some 1600 slides, and in the preparation of the manuscript.

* In the remainder of this paper alkaline phosphatase will at times be referred to as AP.

This work represents a condensation of the thesis submitted to the faculty of the New York University Post-Graduate Medical School in partial fulfillment of the requirements for the degree of Master of Science in Dermatology and Syphilology.

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A review of the literature shows that AP has been demonstrated in many structures of normal skin (Table 1).

The problem of localization of AP activity in relation to nuclear and cytoplasmic components has been the subject of much comment, and many points are far from settled; however, the presence of AP in nuclei and cytoplasm has been demonstrated by centrifugation methods.[26,35,96,97] Most authorities agree that with the methods used some of the AP demonstrated in nuclei and cytoplasm is not actually present in vivo but is demonstrable because of diffusion of the enzyme, of products of hydrolysis, or of activators. Nevertheless, although some false positivity occurs, most of the phosphatase demonstrated is in conformity with its distribution in vivo.[24,41,52,54,64,76,86,91,109,121,123,137]

At the present state of our knowledge, it has been decided to report sites of AP distribution as demonstrated by the Gomori-Takamatsu technique and to await further studies to judge whether the enzyme so demonstrated represents its actual in vivo location.

B. Reported Distribution of AP in Skin Diseases

Two previous reports dealt solely with the distribution of AP in normal and diseased human skin.

The most extensive work is that of Pirilä and Eränkö,[156] who investigated normal skin as well as some 26 dermatologic conditions. This is the basic study of AP in relation to skin disease. Any differences between their findings and the results of the investigations presented here will be pointed out in the discussion.

The second report is that of Fisher and Glick,[56] whose results, along with those of other investigators, are included in Table 2.

Monacelli and Ribuffo[128] found flat serum phosphate curves associated with hyperglycodermia in psoriasis. These authors felt that a disturbance in AP and consequent phosphorylation resulted in hyperglycodermia. Hollander[87] reported high AP activity of capillaries in psoriasis.

TABLE 1.—*The Distribution of Alkaline Phosphatase as Previously Reported in Normal Skin*

Site	Reference	Findings
Stratum corneum	129	Cobalt sulfide deposit reported, but unclear whether false positive precipitate was ruled out
Stratum granulosum	22, 84, 156	Weak staining of nuclei and keratohyalin, but some authors noted even unstained granules simulated weak staining, owing to their optical density
Stratum mucosum	137, 156	No AP activity except during earliest stages of embryogenesis (chick embryo)
Stratum germinativum	56, 145, 156	Most authors report no activity; Newman[145] found AP present
Cutaneous capillaries	56, 68, 145, 156	All workers agree that dermal capillaries show moderate to high AP activity
Dermal connective tissue	137, 145, 156	Most observers found no AP activity; some may occur on longer incubation and occasionally on fibrocytes near certain strongly active elements; intense activity during embryogenesis
Hair	22, 33, 56, 84, 92, 135, 178	All authors agree that the hair papilla shows high activity during the anagen stage and reduced or no activity in catagen and telogen stages; AP reported by some in hair matrix nuclei and external connective tissue sheath; false positive deposit on inner root sheath[145]
Eccrine sweat glands	29, 145, 156, 173	All authors described AP activity in the myoepithelium and acinar cells, and its absence from the sweat ducts
Apocrine sweat glands	28, 29, 136	AP activity was found in the myoepithelium, acinar cells and luminal contents; activity generally less than eccrine glands[29]
Ceruminous glands	135	Myoepithelium and basal portions of ceruminous glands moderate activity whereas apices of acinar cells have little; luminal contents have high false positive deposit but minimum true AP activity
Sebaceous glands	130, 136, 156, 178	Human glands: no AP activity or slight nuclear deposit in stratum germinativum; animal (rat, dog, etc.): moderate AP activity predominantly of stratum germinativum
Arrector pilorum muscle	145, 156	AP absent[145] or slight on long incubation[156]
Stromal mast cells	129, 148	AP activity present in animal (dog, rat) cutaneous mast cell granules
Subcutaneous fat tissue	156	AP activity absent, with rare exceptions

TABLE 2.—*The Distribution of Alkaline Phosphatase, as Previously Reported, in Skin Diseases* *

Disease	Reference	Findings
Acne vulgaris	56	Diffuse deeper staining involving the lymphocytic infiltrate
Eczema	56	AP activity in the perivascular infiltrations; diffuse staining of some fibroblasts in deep cutis
Lupus erythematosus	56	Normal AP distribution
Molluscum contagiosum	126	AP activity in nuclei of basal and prickle cells, and dermis, in relation to the molluscum affected rete pegs; false positive precipitate of inclusion bodies and keratohyaline of stratum granulosum
Papular urticaria	56	Normal AP distribution
Psoriasis	56	Normal AP distribution
Psoriasis	87	High activity of thick walled papillary capillaries
Pyogenic granuloma	56	AP activity observed in new fibroblasts and inflammatory cells
Scar	56	Increased AP activity in relation to fibroblasts, especially in the early phases

* Authors other than Pirilä & Eränkö.[106]

In the treatment of cutaneous tuberculosis, it has been postulated that vitamin D acts by increasing the AP activity in the skin. Alkaline phosphatase activators (e. g., magnesium) enhance vitamin D action, whereas AP inhibitors (e. g., cysteine) diminish vitamin D effectivity (Charpy[32]).

In the course of Amos'[4] studies of factors influencing the "spontaneous" inactivation of the herpes simplex virus, he found that AP of the tissue in which the virus was propagated played a significant role in this inactivation. By addition of purified AP to suspensions of the virus the rate of deterioration was noticeably increased. Greater survival of the virus occurred in the presence of AP inhibitors. Finally, he showed that purified AP added to herpes simplex virus brought about loss of infectivity.

Beryllium has been shown to inhibit AP. It has been suggested that beryllium displaces magnesium (an AP activator) and thereby inactivates AP. It is postulated that this leads to a granulomatous response. Klemperer[104] reported the inhibition of AP by Be concentrations lower than 10^{-6}M. Grier[80] showed that this inhibition is partly reversible by adding Mg ions (also Aldridge[3]), but the inhibitory effect of Be is more marked than the activating effect of Mg. The ammonium salt of aurintricarboxylic acid and citrate reverse the Be inhibition. Aldridge[3] suggested that there exists a competitive relationship between Be and Mg for AP.

TABLE 3.—*Alkaline Phosphatase as Previously Reported in Human Neoplastic Diseases*

I. Tumors showing AP activity equal to or greater than parent tissue	
Adenocarcinoma (intestine)	Greenstein [78]
Adenocarcinoma (kidney)	Manheimer & Seligman [113]
Adenocarcinoma (ovary)	Manheimer & Seligman [113]
Astrocytoma	Landow [111]
Carcinoma solidum (skin)	Pirilä & Eränkö [106]
Fibroadenoma (breast)	Kabat & Furth [98]
Oligoblastoma	Landow [111]
Meningioma	Landow [111]
Metatastic lesion to bone	Greenstein [78]
Oligodendroglioma	Landow [111]
Osteogenic sarcoma	King [103]
II. Tumors showing AP activity less than parent tissue	
Adenocarcinoma (intestine)	Manheimer & Seligman [113]
Adenocarcinoma (uterine)	Atkinson & Gusberg [9]
Carcinoma spinocellulare	Pirilä & Eränkö [106]
Malignant melanoma	Manheimer & Seligman [113]
Mammary carcinoma	Greenstein [78] & Franseen [69]
Prickle-cell epithelioma	Manheimer & Seligman [113]

C. Reported AP Activity in Human Neoplastic Diseases

It has been demonstrated that tumors arising in tissues normally having AP present may show either increase, normal quantities, decrease, or lack of this enzyme. In addition, tumors of elements which normally show no AP may be associated with great quantities of demonstrable enzyme. Also, histologically similar tumors of the same organs obtained from different patients, or even from the same patient, may show variation in the degrees of activity. Some primary neoplasms show AP activity quite different from that of their metastatic lesions. Finally, benign tumors of similar structures may differ in their activity from their malignant counterparts.

The findings concerning AP distribution in human neoplastic diseases are summarized in Table 3.

D. Identification of Alkaline Phosphomonoesterases

The technique used in these experiments [76] is a modification of the original methods of Gomori [66] and Takamatsu,[184] in which the tissue is incubated in the presence of a mixture which acts as an indicator of the enzyme.

Five essential ingredients are required: (1) the substrate: β-glycerophosphate; (2) the activator: magnesium ions; (3) the phosphate precipitator: calcium ions; (4) the indicators: cobalt and sulfide ions, and (5) the buffer: sodium barbital. The magnesium ions in the incubation mixture activate the reaction.

The sodium barbital buffer adjusts the pH to about 9.2, which is the optimum pH for AP activity, and it also prevents false positive reactions which may be due to the presence of other enzymes, such as acid phosphatase, which at pH 9.2 are inactivated.

The chemical reactions taking place in the Gomori-Takamatsu technique are summarized as follows:

$$(a)\ \text{Sodium } \beta\text{-glycerophosphate} \xrightarrow{\text{AP}} \text{glycerine} + \text{phosphate ions}$$

$$(b)\ \text{Phosphate ions} + \text{calcium ions} \xrightarrow[\text{pH } 9.2]{} \text{calcium phosphate}$$

$$(c)\ \text{Calcium phosphate} + \text{cobalt nitrate} \longrightarrow \text{cobalt phosphate}$$

$$(d)\ \text{Cobalt phosphate} + \text{ammonium sulfide} \longrightarrow \text{cobalt sulfide}$$

Consequently, sites of AP activity are demonstrated by deposits of black cobalt sulfide.

E. Factors Influencing AP Activity, Its Identification and Localization

The factors known to influence the histochemical demonstration of AP are numerous. An understanding of their roles is important for the interpretation of the results obtained. These factors are presented in Table 4.

II. Material and Methods

The results herein reported are based on the study of 1435 sections of human skin obtained from 253 specimens.

Details of the technique followed on all slides are outlined as follows:

A. Fixation (50 Hours).—Fresh tissues were chilled (4 C) for 24 hours. After the specimens gained firmness they were trimmed down to 3 mm. thickness. Dehydration was accomplished by two changes of absolute ethyl alcohol, 12 hours each, followed by two changes of chloroform, 1 hour each.

B. Infiltration (One Hour).—The tissues were placed into an open incubator dish of soft paraffin (M. P. 48 C) at 56 C for 15 minutes, then for 35 minutes in a vacuum bottle containing soft paraffin under reduced pressure, and finally into an open dish of soft paraffin for 10 minutes.

C. Imbedding.—The specimens were imbedded in hard paraffin (M. P. 56 C).

D. Sectioning.—Sectioning was performed at 4μ. The sections were floated on lukewarm water, mounted on albumin glycerine slides, and dried (56 C for 10 minutes, then 37 C for 12 hours).

TABLE 4.—*Factors Influencing AP Activity, Its Identification and Its Localization* *

I. False negative findings (i.e., negative findings in spite of known presence of enzyme) may result from

1. Enzyme inactivation by fixation or imbedding
2. Enzyme inactivation due to prolonged storage
3. Diffusion of enzyme
4. Subthreshold activity
5. Rate of phosphate ion production
6. Presence of inhibitors
7. Absence of activators
8. Insufficient incubation time
9. Incorrect pH range

II. False positive findings (i.e., similar to genuine reaction but not due to enzyme) may result from

1. Pigments similar to cobalt sulfide (e.g., hemosiderin)
2. Performed calcium
3. Absorption of cobalt on certain structures (e.g., muscle, keratin)
4. Spontaneous disintegration of substrate
5. Bacterial hydrolysis of substrate

III. False localization (i.e., enzyme appearing at site different from its exact in vivo location) may result from

1. Diffusion of enzyme
2. Diffusion of products of hydrolysis

* After Gomori. [14,16]

E. Incubation (Four Hours).—The slides were carried through xylene (one minute, three times), absolute alcohol (one-half minute, twice), 0.5% collodion in alcohol-ether (two minutes), 80% alcohol (one minute), and, finally, distilled water (three minutes).

The specimens were then incubated at 37 C for four hours in the following mixture:

Sodium β-glycerophosphate (3% aqueous) 100.0 ml.
Calcium chloride (2% aqueous) .....250.0 ml.
Magnesium chloride (10% aqueous) .....2.5 ml.
Sodium barbital (powder) .....7.5 gm.
Distilled water.....to make.....500.0 ml.

If the mixture became turbid it was filtered before use. The pH, measured on several occasions, was between 9.2 and 9.3.

F. Calcium Phosphate Precipitation.—After incubation the slides were treated as follows:

1. Running tap water, one minute
2. 2% cobalt nitrate,† five minutes
3. Running tap water, one minute
4. Dilute yellow ammonium sulfide solution (10 drops solution of yellow ammonium sulfide ‡ in 200 ml. distilled water), five minutes
5. Running tap water, five minutes.

G. Stains and Counterstains.—All specimens were stained as follows:

1. Hematoxylin and eosin. Delafield hematoxylin and aqueous eosin (pH 6.3). Both incubated and nonincubated slides were similarly stained.
2. Carmine. Incubated specimens were counterstained with lithium carmine; other counterstains (eosin, metanyl yellow, picric acid) were not found to be as satisfactory.
3. Special stains. Other stains were used when indicated in order to identify special structures.

† $Co(NO_3)2\ 6H_2O$, Merck & Company, No. 7249.
‡ Fisher Scientific Company, No. S-314.

H. Mounting.—Slides were mounted with Damar-xylene solution.§

I. Controls.—Controls on 114 slides were carried out in the following manner:

1. Five specimens were incubated for varying lengths of time (½, 1, 2, 3, 4, 5, 6, 12, 24 hours).[39,51]
2. Forty-three specimens were incubated without the glycerophosphate substrate.
3. Eighteen specimens were heated to 80 C for 10 minutes (thus inactivating the enzyme) and then treated as described above under "Incubation" and "Calcium Phosphate Precipitation."
4. Twelve specimens were simply deparaffinized and cover slips were applied, in order to observe preformed pigment.
5. Several representative specimens (deparaffinized and taken to water) were treated in one or all of the following manners:
   (*a*) no incubation; slide deparaffinized
   (*b*) no incubation; cobalt nitrate (five minutes)
   (*c*) no incubation; ammonium sulfide (five minutes)
   (*d*) no incubation; cobalt nitrate (five minutes); ammonium sulfide (five minutes)
   (*e*) 1% calcium chloride solution (two minutes); cobalt nitrate (five minutes)
   (*f*) 1% calcium chloride solution (two minutes); ammonium sulfide (five minutes)
   (*g*) 1% calcium chloride solution (two minutes); cobalt nitrate (five minutes); ammonium sulfide (five minutes)
   (*h*) 1% calcium chloride solution (four hours) incubated at 37 C then exposed to cobalt and sulfide ions in the routine manner

§ Harman-Leddon Company.

(*i*) incubation with regular incubation mixture (four hours); cobalt nitrate (six minutes)

(*j*) incubation with regular incubation mixture (four hours); ammonium sulfide (five minutes)

(*k*) incubation with regular incubation mixture (four hours); cobalt nitrate (five minutes); ammonium sulfide (five minutes)

J. Interpretation.—Cobalt sulfide, seen as fine granular brown to black precipitate, indicated sites of AP, with the specific exception of the false positive findings discussed below.

In the body of the paper the terms AP activity, deposit, and precipitate will all refer to true enzymatic action, unless otherwise mentioned.

III. Results

A. Controls

The findings in controls will be presented first, because interpretation of subsequent slides can have meaning only in the light of knowledge of these variables.

1. *False Positive Reactions.*—Precipitation of cobalt sulfide dependent upon factors other than AP action was found principally in two sites.

The most striking was the inner root sheath of the hair follicles, which showed an opaque, intensely black cobalt sulfide deposit. This precipitate, much darker than any precipitate due to AP activity, began in Henle's layer just distal to the hair bulb, considerably proximal to the zone of keratinization. Immediately distal to the zone of keratinization (i. e., beyond the keratohyaline granules) Huxley's layer began to show this deep black precipitate. Both layers of the inner root sheath were intensely stained, beginning proximally, as stated above, and continuing distally to the termination of these layers near the follicular orifice (Fig. 8).#

The second important site of false positive precipitation was found (but with no such regularity) in soft keratin. This was especially the case in hyperkeratosis (Fig. 12) and parakeratosis, but occasionally it was seen in specimens showing normal horn.

Occasional false positive precipitates were seen in hair medullae. Control sections showed a grayish "haze," which often outlined nuclear and cytoplasmic membranes of all structures and of inflammatory cells, so that even without counterstain one could identify most tissue elements. However, this fine gray deposit could hardly be confused with the brown to black precipitate seen in areas of AP activity.

False positive faint deposits were seen on keratohyalin granules, degenerated elastica, "colloid," and at sites of preformed calcium.

The above interpretations were based on the following control studies:

(*a*) In specimens incubated for lengths of time varying from ½ to 24 hours, the conclusion was reached that a 4-hour incubation period was the most satisfactory. A period shorter than this did not accentuate all areas of weaker AP activity, while a longer incubation produced a disproportionate increase of the deposit in areas surrounding high concentrations of AP (possibly due to diffusion of enzyme or products of hydrolysis).

Specimens incubated for one-half hour showed only a mild to moderate deposit of cobalt sulfide in sites of AP activity. Specimens incubated between 2 and 6 hours showed a moderate to heavy deposit, whereas in those incubated 12 to 24 hours it was heavy to intense.

Fisher and Glick [56] and Pirilä and Eränkö [156] used longer incubation (12 to 24 hours), which may have been responsible for some of the differences between their findings and those reported here.

In summary, the longer the incubation (up to 24 hours), the more intense the cobalt sulfide precipitation. Four hours seemed to be the optimum incubation period.

(*b*) Forty-three slides were incubated without glycerophosphate in the mixture, and in the absence of substrate no AP activity could be demonstrated. Cobalt sulfide was seen only as false positive findings in areas described above.

‖ These findings corroborate those of Pirilä and Eränkö.[156]

The illustrations will be found in a group after the references.

(*c*) Eighteen slides were heated at 80 C (10 minutes), then treated as regular incubated AP slides. These slides had cobalt sulfide only at sites of false positive reactions. No AP was demonstrable, since the enzyme was heat-inactivated.

(*d*) Deparaffinized sections showed pigments including melanin, hemosiderin, and an orange-brown stain seen in the keratin of some hyperkeratotic lesions. A few larger vessel walls, and the erythrocytes within their lumens, showed a yellowish-brown pigmentation.

The results of the series of controls carried out in deparaffinized sections are summarized in Table 5.

TABLE 5.—*Alkaline Phosphatase Controls*

	Procedure	Results
(a)	Deparaffinized section (no incubation)	Preformed pigments seen
(b)	Cobalt ion only (no incubation)	No precipitate
(c)	Sulfide ion only (no incubation)	No precipitate
(d)	Cobalt ion followed by sulfide ion (no incubation)	No precipitate
(e)	Calcium ion followed by cobalt ion	No precipitate
(f)	Calcium ion followed by sulfide ion	No precipitate
(g)	Calcium ion (2 min.) followed by cobalt ion and sulfide ion	False positive precipitate as described in text
(h)	Calcium ion (4 hr.) followed by cobalt ion and sulfide ion	False positive precipitate as described in text
(i)	Incubation mixture (4 hr.) followed by cobalt ion	No precipitate
(j)	Incubation mixture (4 hr.) followed by sulfide ion	No precipitate
(k)	Incubation mixture (4 hr.) followed by cobalt and sulfide ion	False and true AP activity

Summary: The optimal incubation period was found to be four hours. False positive precipitates were found in substantial measures in relation to two elements: the inner root sheath of hair follicles and soft keratin.

Light false positive deposit was seen on keratohyalin of the stratum granulosum, degenerated elastica, "colloid," and in the hair medulla. Smooth muscle showed a very faint deposit, to give a yellowish gray hue. A widespread fine granular deposit was also seen over the entire sections, mainly on nuclear and cytoplasmic membranes.

Specimens exposed (for four hours) to calcium chloride solution alone (i. e., without the glycerophosphate substrate) and then treated with cobalt and sulfide ions showed only areas of false positive precipitates. Exposure of similar specimens to calcium ions for a period of two minutes only was sufficient to produce the total precipitates of false positive reactions. All these ions (calcium, cobalt, and sulfide) were necessary to produce the black cobalt sulfide precipitate of enzymatic or false positive origin. In the absence of one or more of these ions, cobalt sulfide precipitate did not occur.

B. Distribution of Alkaline Phosphatase in Normal Human Skin

Table 6 summarizes the findings in 29 biopsy specimens from various areas of the body.

1. Stratum corneum: Faint false positive deposits were seen in the stratum corneum of 11 specimens. One specimen, from the sole, showed intense false positive precipitation (Fig. 12).

2. Stratum lucidum: One specimen had a faint gray homogeneous deposit in the stratum lucidum. No control studies were made to decide whether this deposit was due to AP activity or whether it represented a false positive reaction.

3. Stratum granulosum: Deparaffinized sections showed colorless keratohyaline granules in the stratum granulosum which were recognizable on account of their refractivity. Faint false positive deposit was seen in relation to these granules in control section (i. e., those incubated without glycerophosphate substrate and those merely subjected to calcium, cobalt, and sulfur ions). Specimens incubated with the substrate had precipitates on the keratohyaline granules identical with those seen in the control sections, indicating that these granules had no true AP activity.

TABLE 6.—*Distribution of Alkaline Phosphatase in Normal Skin* *

AREAS	Epidermis			Cutis				Hair				Sweat Glands: Eccrine					Sweat Glands: Apocrine					Sebaceous Glands		Adipose Tissue		Comment
	Stratum Corneum	Prickle Layer	Basal Layer	Connective Tissue	Capillaries	Large Vessel Endothelium	Papilla	Outer Root Sheath	Inner Root Sheath	Shaft-Cortex	Shaft-Medulla	Surrounding Vessels	Acinar Cells	Myoepithelium	Contents	Duct	Surrounding Vessels	Acinar Cells	Myoepithelium	Contents	Duct	Stratum Germinativum	Surrounding Vessels	Interlobar Septae	Inflammatory Infiltrate	
Scalp	0	0	MG	0	2+	—	3+	0	FP	0	0	1+	1+	1+	0	0	—	—	—	—	—	0	1+	—	—	
Temple	0	0	MG	0	1+	1+	3+	0	FP	0	0	2+	1+	1+	0	0	—	—	—	—	—	0	1+	0	—	
Forehead	FP	0	MG	1+	2+	1+	3+	0	FP	MG	—	2+	1+	1+	0	0	—	—	—	—	—	0	2+	0	—	
Cheek	0	0	MG	0	2+	1+	3+	0	FP	0	0	2+	2+	2+	0	0	—	—	—	—	—	0	2+	1+	—	
Face	0	0	MG	0	1+	0	3+	0	FP	0	0	—	—	—	—	0	—	—	—	—	—	1+	1+	0	—	
Mucosa	—	0	0	0	3+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Mucosa	0	0	0	0	3+	2+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Mucosa	—	0	0	0	2+	2+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Neck	FP	0	MG	0	1+	1+	1+	3+	0	FP	MG	—	2+	1+	1+	0	—	—	—	—	—	0	2+	1+	—	Basophilic degeneration of elastica shows false positive precipitate
Axilla	FP	0	MG	0	2+	—	0	0	FP	FP	0	—	—	—	—	—	2+	1+	1+	1+	0	—	—	—	—	AP concentrated in cytoplasm at free pole of apocrine acinar cells
Arm	FP	0	MG	0	2+	1+	—	0	FP	0	0	2+	1+	1+	0	0	—	—	—	—	—	—	—	0	1+	
Wrist	0	0	MG	0	2+	1+	3+	0	FP	0	0	1+	1+	1+	0	0	—	—	—	—	—	—	—	—	—	
Scapular	0	0	MG	0	2+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Scapular	FP	0	MG	0	2+	1+	3+	0	FP	MG	—	2+	1+	1+	0	0	—	—	—	—	—	0	2+	—	—	
Ant. thorax	FP	0	MG	0	1+	1+	—	0	FP	—	—	2+	1+	2+	0	0	—	—	—	—	—	0	1+	1+	—	
Pos. thorax	0	0	MG	0	1+	0	—	0	FP	—	—	1+	1+	1+	0	0	—	—	—	—	—	0	1+	0	—	
Pos. thorax	FP	0	MG	0	2+	1+	—	0	FP	—	—	2+	1+	2+	0	0	—	—	—	—	—	0	2+	0	—	
Abdomen	FP	0	MG	0	2+	1+	—	0	FP	MG	1+	2+	2+	2+	0	0	1+	1+	2+	1+	0	1+	1+	0	—	Sweat gland and duct nucleoli show intense AP activity
Abdomen	FP	0	MG	0	2+	1+	3+	0	FP	MG	1+	2+	2+	2+	1+	0	—	—	—	—	—	0	2+	0	—	Sweat gland and duct nucleoli show intense AP activity
Abdomen	0	0	MG	0	2+	1+	—	0	0	0	0	1+	1+	1+	0	0	—	—	—	—	—	—	—	—	—	Very few vessels, especially papillary capillaries
Coccygeal	FP	0	MG	0	2+	—	—	0	FP	MG	—	2+	2+	2+	0	0	—	—	—	—	—	0	2+	0	—	
Thigh	0	0	MG	0	1+	0	—	0	—	—	—	—	—	—	—	0	—	—	—	—	—	—	—	—	—	Very little activity in papillary capillaries
Leg	0	0	MG	0	2+	1+	—	0	FP	MG	0	2+	1+	1+	0	—	—	—	—	—	—	—	—	—	—	
Leg	0	0	MG	0	2+	—	—	—	—	—	—	2+	1+	1+	0	—	—	—	—	—	—	—	—	—	—	
Leg	FP	0	MG	0	2+	1+	—	—	—	—	—	2+	1+	2+	0	0	—	—	—	—	—	0	1+	0	—	
Sole	FP	1+	1+	1+	3+	1+	—	—	—	—	—	2+	2+	2+	0	0	—	—	—	—	—	—	—	1+	—	Vasa vasorum 1+; stratum corneum, heavy false positive precipitate
†?	0	0	0	1+	—	1+	3+	0	FP	MG	—	—	—	—	—	—	—	—	—	—	—	0	0	0	—	
†?	0	0	0	1+	1+	1+	3+	0	FP	MG	—	—	—	—	—	—	—	—	—	—	—	0	1+	1+	—	
†?	FP	1+	0	1+	2+	1+	3+	0	FP	MG	—	—	—	—	—	—	—	—	—	—	—	0	1+	1+	—	

*—, structure absent from specimen; 0, no deposit, but structure present; 1, light brown deposit = slight AP activity; 2, dark brown deposit = moderate AP activity; 3, black deposit = intense AP activity; FP, false positive cobalt sulfide precipitation; MG, melanin granules.

† Premature infants.

4. Stratum malpighii and basal layers: No precipitate was seen in the prickle or basal layers.

5. Connective tissue: Slight precipitate was seen on collagen bundles in three specimens, but in general no AP activity was found. A notable exception to this was a mild activity found in the more cellular connective tissue elements of premature infants.

6. Vessels: Papillary capillaries (Figs. 1 and 2) invariably showed AP activity, which was seen in the nuclei as well as in the cytoplasm of the endothelium. Nuclear AP was often concentrated toward the periphery, whereas cytoplasmic activity was more diffuse. Only rarely were capillaries seen without deposit. The walls of larger vessels in the deeper cutis were usually negative but occasionally manifested minimal to moderate endothelial activity (Fig. 12). In addition, some of the largest vessels showed light activity of the vasa vasorum.

7. Smooth muscle: A diffuse slight, yellowish-gray false positive deposit was commonly observed.

8. Nerves: No activity was demonstrated in any section.

9. Sebaceous glands: Several specimens had a minimal deposit in the nuclei and cytoplasm of cells in the germinative layer. At times the nucleoli of cells toward the periphery of the sebaceous gland were darkly stained, but as the cells were followed to the center their nucleoli showed diminution of activity, so that at the center, where the cells began to disintegrate, even the nucleoli contained no deposit. Surrounding the sebaceous glands there were networks of fine capillaries with substantial AP activity (Fig. 6).

10. Eccrine sweat apparatus: In the acinar portion of the eccrine apparatus the secretory cells were found consistently to have moderate to heavy AP activity in the cytoplasm furthest from the lumen (Figs. 11 and 12). Occasionally the precipitate was also seen on the nuclei and nucleoli, and rarely, in the cytoplasm nearest the lumen. There was no deposit within the lumens. The myoepithelial cells showed AP activity in the cytoplasm, nuclei, and nucleoli. The deposit was usually heavier in the myoepithelial cells than in the acinar cells. Vessels showing marked activity in their endothelium formed a rich network around the eccrine glands. The basement membrane had a moderate deposit. The eccrine ducts showed no AP activity. At times the ducts were surrounded by AP-active capillaries.

11. Apocrine sweat apparatus: The distribution of AP in the apocrine glands differed from that in the eccrine glands, and the over-all activity was invariably less (Fig. 10).

The distribution differed in two ways. First, the apocrine gland secretory cells, instead of having the deposit only in the peripheral portion of the cells, showed bipolar activity, so that the deposit was almost as intense in the luminary portion. The area between the poles often had no activity, though occasionally a false deposit was seen in the nuclei, nucleoli, and cytoplasm.

Secondly, the contents within the lumens of apocrine glands showed faint to moderate deposits such as were not found in eccrine glands.

Like the eccrine glands, the apocrine glands showed AP activity of the myoepithelium, the basement membrane, and the periglandular vessels. The ducts showed no activity, but moderate deposit was seen in the vessels adjacent to them.

12. Mucous glands (buccal): Faint enzymatic activity was seen in some, but not all, of the peripheral acinar cells (nuclear deposit greater than cytoplasmic).

13. Hair: The most striking AP activity in human skin was found in the hair papilla (Fig. 8). Here there was almost consistently found a dense enzymatically produced precipitate. Often reaction was so intense that one could not define individual papillary components. Similar intensity was seen in the hair sac (connective tissue sheath) surrounding the bulb. Above the bulb, the loose stroma surrounding the hair follicle showed no deposit other than that seen in occasional capillaries. Some specimens pre-

sented unusual capillary networks, with activity, surrounding the entire hair apparatus. Vellus (lanugo) hair papillae generally situated in the cutis evinced consistently greater degrees of activity than terminal (large) hair papillae located in the subcutis.

The false positive precipitate of the inner root sheath and medulla of the hair shaft has been described under controls. The outer root sheath was devoid of cobalt sulfide. The yellow-brown color of melanin in the hair bulb and in the cortex of the hair shaft could be distinguished from the brown-black cobalt sulfide. The hair medulla showed slight (false positive) precipitate in two specimens. The hair bulb matrix cells generally showed minimal AP activity near the central hair papilla.

14. Subcutaneous fat: In four specimens a fine precipitate was seen in the connective tissue and vessels of the subcutaneous interlobar septae. However, generally speaking, there was no AP activity of the septae, and none was found in the fat cells themselves. There was only mild activity of the capillaries coursing through the interlobar septae.

C. Alkaline Phosphatase in Diseases of Human Skin

Mention will be made only of AP distributions differing from those seen in normal tissues.

Histopathologic descriptions of the specimens will be given only if necessary to illustrate certain features.

An attempt has been made as far as possible to group together diseases which are etiologically, histogenetically, or otherwise related to each other.

(1) *Inflammatory Dermatoses.*—Erythema Nodosum (One Specimen): There was moderate AP activity of the infiltrate and connective tissue of the subcutis, apart from numerous capillaries with activity throughout the inflammatory zone; deposit was concentrated on the nuclei of fibroblasts. There was moderate AP activity of the collagen fibers of the lower cutis.

Chronic Eczema (One Specimen): There was considerable activity of the exocytotic cells (mostly nuclear). The prickle cells (some of which were disintegrating) near these inflammatory cells also showed some activity; those farther away showed only nucleolar deposit. The basal cells showed slight perinuclear deposit. There were AP-active dilated capillaries in the papillae, surrounded by banal infiltrates, showing AP activity, also predominantly nuclear. In one area of the cutis the connective tissue near inflammatory cells had a moderate precipitate. An infiltrate around the sweat glands showed moderate activity.

Distinctive Exudative Discoid and Lichenoid Chronic Dermatosis (One Specimen): The epidermis showed moderate activity localized to groups of exocytic and disintegrating epidermal cells found in vesicles. There was an increase in the number of papillary capillaries, all highly active. Some thickened vessels of the upper cutis had AP-active endothelium. Except for mild nuclear reaction there was no deposit in the inflammatory infiltrate.

Drug Eruption (One Specimen): There was considerable perivascular cellular infiltrate, with increase of the capillaries in the upper corium and midcorium. These capillaries showed AP activity in the endothelial cytoplasm and nuclei, most markedly in the nucleoli and at the periphery of the nuclei. The inflammatory cells showed light deposit in the nuclei and more in the nucleoli. The intensity of the precipitate was greatest near the capillaries.

Neurodermatitis (Three Specimens): All specimens showed papillary vessels with moderate AP activity. Two specimens showed mild activity in groups of exocytotic elements. One specimen had a faint precipitate in the nuclei of perivascular inflammatory cells.

Lichen Corneus Hypertrophicus (One Specimen): No variation from the normal was seen.

Fox-Fordyce Disease (Two Specimens): Alkaline phosphatase activity was that of normal axillary skin. The dilated tubules

showed a minimal deposit, more consistent with that of apocrine than with that of eccrine glands. Periductal vascular AP activity was increased.

Psoriasis (Two Specimens): There was a slight increase in papillary capillaries, which were highly active. A mild deposit was seen in the inflammatory cells surrounding them. One specimen showed pronounced activity in and surrounding several microabscesses (Fig. 3), not only of the exocytotic elements but also of adjacent epidermal cells. The cytoplasm of the latter cells contained numerous minute black keratohyalin granules in addition to diffuse cytoplasmic and nuclear deposits.

Parapsoriasis (One Specimen): This showed AP-positive papillary capillaries, surrounded by inflammatory cells with some nuclear deposit. The contents of a subcorneal vesicle showed a moderate precipitate.

Lichen Planus (Four Specimens): AP activity was seen in capillaries of the upper cutis. Some of the inflammatory cells near them showed mild deposit.

Lupus Erythematosus (One Specimen): AP activity was mild in the increased perifollicular vessels and in some inflammatory cells in these areas.

(2) *Bacterial and Spirochetal Diseases.*—Folliculitis (One Specimen): There was moderate AP activity in the endothelium of the increased capillaries. The cellular infiltrate near them showed a deposit, greater in the nuclei and less in the cytoplasm.

Lupus Vulgaris (One Specimen): The central (epithelioid) portions of the tubercles were devoid of AP activity, while the surrounding lymphocytic zones showed heavy reaction. The activity in these outer zones was seen in vessels, fibrocytes, and lymphocytes. Langhans cells showed no activity. In the mid cutis and lower cutis numerous thin-walled vessels, not related to tubercles, were seen with heavy deposit.

Erythema Induratum (One Specimen): Throughout the subcutis there was a tubercular infiltrate. The caseated areas were devoid of AP activity except for minimal nuclear deposit in the inflammatory and necrotic cells (Fig. 14). Toward the more definite tubercular reaction at the periphery of the tubercles there was gradual increase in deposit, finally showing very high activity. Activity was seen in the inflammatory cells, on the connective tissue of the fat trabeculae, and in capillaries. The endothelium of thick subcutaneous vessels showed marked activity. No deposit was seen in the Langhans cells present. There was a general activity throughout the cutis on the collagen fibers and lining the spaces between them. Nuclei of fibroblasts and histiocytes in the upper cutis showed moderate activity.

Nodular Tertiary Syphilis (One Specimen): All vessel lumens were narrowed. Those of the upper cutis were not thickened, whereas those in the deeper parts had distinctly thickened walls, but all were active. The perivascular infiltrates were negative.

Syphilitic Keratoderma Punctatum (One Specimen): The specimen showed a parakeratotic mass pressing into and thinning the epidermis, causing flattening of rete pegs. There were intense perivascular infiltrates of lymphocytes, histiocytes, and plasma cells. The vessels showed thickened walls. There was stromal proliferation surrounding the eccrine glands. The parakeratotic plug, and to a lesser extent the adjacent horny layer, showed a false positive precipitate. The thickened vessels and surrounding cells showed moderate activity. Intense activity in the proliferating stroma around the sweat glands is noteworthy.

(3) *Viral Diseases.*—Molluscum Contagiosum (Two Specimens): Both specimens showed heavy AP activity in inflammatory cells (mainly nuclear) and in the stroma of the upper cutis. The active capillaries of the papillae between the affected rete pegs were compressed. The basal layer and a few rows of adjacent prickle cells showed mild activity, greatest in their nucleoli, less in the nuclei, and least in the cytoplasm. The keratohyalin of the granu-

lar layer showed definite AP activity (as substantiated by control slides not using the substrate). The inclusion bodies themselves showed mild enzymatic reaction. It was apparent that the older (i. e., the more superficial) the molluscum body, the greater its AP positivity. Heavy deposits were seen near the surface where affected cells were disintegrating.

Verruca Vulgaris (Four Specimens): The thickened stratum corneum showed considerable false positive precipitate in all sections. There was an increase in the papillary capillaries, with considerable AP activity. One specimen had prominent granules in the stratum granulosum, but, unlike those seen in molluscum contagiosum, these granules showed no precipitate. One specimen had intense AP activity in relation to the fibroblastic reaction in the cutis.

(4) *Granulomas of Unknown Origin.*—Sarcoid (One Specimen). The tubercles of sarcoid were devoid of AP activity. There was a general sparsity of vessels, so that that AP-stained sections showed fewer capillaries than would be expected in normal skin. Epithelioid, giant, and lymphocytic cells were devoid of activity.

Granuloma Annulare (Two Specimens): Many connective tissue fibers surrounding areas of granular degeneration showed mild AP activity, whereas the degenerated tissue itself had no activity. One specimen revealed a minimal increase in the number of vessels, thickening of their walls, and strong endothelial AP activity. The cellular infiltrate showed mild or no activity.

Facial Granuloma (One Specimen): The specimen could not be classified more definitely. Sections showed a dense dermal infiltrate of lymphocytes and histiocytes, some giant and multinucleated cells. Hardly any activity was demonstrable other than that of the vessels within the infiltrate. The giant cells showed considerable activity of their nuclei, less in their cytoplasm, thereby standing out from the other cell types.

Granuloma, Nonspecific Foreign Body Type (Three Specimens): Specimen 1 showed a cutaneous and subcutaneous granulomatous response composed of numerous capillaries, infiltrates of lymphocytes, leukocytes, foreign body giant cells, and considerable fibroblastic reaction. There were central necrosis and mild deposits of hemosiderin. Within the necrotic area the AP activity was diminished, but this was surrounded by a wide zone of intense activity in relation to three elements: vessels, connective tissue, and cellular infiltrate. The vessels had moderately thickened walls, and some showed endothelial proliferation with substantial deposit. The most intense activity was found in relation to the fibroblasts, where the deposit was apparently concentrated on the surface of these cells. The inflammatory infiltrate had moderate, mainly nuclear, activity.

Specimen 2 showed intense foreign body and giant-cell reaction in the subcutis. Polariscopy revealed birefringent crystals within the giant cells. Moderate AP activity was found in relation to fibroblasts. Mild activity (mostly nuclear) was seen in the banal inflammatory cells; the giant cells (and the crystals which they contained) showed little or no activity.

Specimen 3 showed a granulomatous response in the cutis and subcutis and vessels with thickened walls, some showing endothelial proliferation. Alkaline phosphatase activity was intense in sweat glands, fibroblasts, inflammatory cells, endothelium of vessels, and around the connective tissue in the granulomatous area. Little or no activity was seen in sweat ducts or in the walls of the thickened vessels, causing them to stand out as light areas within the intensely black background. Adipose trabeculae showed marked activity.

(5) *Miscellaneous Diseases.*—Alopecia Areata (Three Specimens): Serial sections showed that some but not all of the hair papillae lacked AP activity. This was most pronounced in early (catagen?) phases of hair loss. Some follicles which showed no activity also manifested shortening of the walls, abnormal relationship of the papillae

to the hair bulbs, or a lack of matrix cells to "cap" the dermal papillary structures. Nevertheless, two specimens also contained some hair papillae which were structurally mature (anagen stage?) and AP-positive. In addition, lanugo-like follicles with highly active papillae were situated in the cutis in all specimens.

The lack of AP activity of certain hair papillae in alopecia areata was in contrast to serial sections of normal skin, where virtually all hair papillae showed moderate to pronounced activity.

Senile Elastosis (Three Specimens): The upper cutis showed advanced changes, representing a combination of collagen and elastica degenerations. The midcutis showed degenerative changes only of elastica. False positive cobalt sulfide precipitate was noted, more pronouncely in degenerated elastica of the midcutis than in the collagen-elastica combination of the upper cutis.

Scleroderma (Two Specimens): There was a remarkable decrease in vascularity compared with normal tissue. Those vessels present stained less intensely and were of smaller caliber than normal. There was a decrease of adnexae. A mild perivascular infiltrate showed slight activity.

Colloid Pseudomilium (One Specimen): Heavy false positive precipitate was observed in three areas: the stratum corneum, the basophilic connective tissue (both collagen and elastic as evaluated with hematoxylin and eosin staining), and the homogenized "colloid" substance. Few AP-active vessels were seen coursing through "colloid" masses.

Riehls' Melanosis (One Specimen): Other than accentuation of the melanin pigment granules in the melanophages of the upper cutis, no abnormal findings were encountered.

Poikiloderma Atrophicans Vasculare (One Specimen): Activity was seen in the endothelium of the numerous dilated vessels of the upper cutis, with some deposit nearby in the inflammatory cells. Scattered deposit was seen lining some of the collagen bundles.

Vitiligo (One Specimen): There were no changes in AP distribution different from normal.

(6) *Diseases of the Vascular System.*— Senile Purpura (One Specimen): Hematoxylin and eosin staining showed extravasation of erythrocytes into the upper cutis, mild inflammatory infiltration, and disruption of vessel walls. There was almost complete lack of AP activity of the ruptured vessels in the upper cutis. Heavy deposit was seen in the subcutaneous fat trabeculae.

Hemangioma (Eight Specimens): One could not predict which hemangioma would show AP activity. Although the histopathologic pictures with hematoxylin and eosin might be similar in two specimens, the vessels of one would show no activity while the other would have intense AP positivity. Of the eight specimens, two showed almost no activity in the vessels. In the other six specimens, which showed mild to intense activity, most of the deposit was seen in the walls of the smaller vessels, in contrast with the usually light activity of the larger and dilated blood channels (Fig. 4). Four specimens showed extramural endothelial proliferation with AP activity in various measures (mildly to moderately intense). Inflammatory components, when present, showed activity mainly in their nuclei. Some vessels showed deposit in thrombi. In two specimens moderate activity was seen in fibroblasts between the vessels.

Lymphangioma (One Specimen): In contrast to findings in the blood vessels of most hemangiomas, there was in this single lymphangioma a complete lack of AP activity of the endothelium of the dilated lymphatics. Blood vessels near the lymph spaces showed normal AP activity.

Granuloma Pyogenicum (Seven Specimens): The AP activity and distribution depended upon the duration ("maturity") of the pathologic process. In general, early lesions showed intense activity of both vascular and cellular components (Fig. 5).

Specimens showing endothelial proliferation and beginning fibrosis usually had greater activity in the fibroblastic areas (nuclear deposits being generally more intense than cytoplasmic). Proliferating endothelial cells often showed little or no activity. In "older" lesions, showing collagen formation, less activity was usually seen, while stroma of mature collagen was devoid of activity. In all lesions most vessels were highly positive, but there were always some which showed little or no activity. In general the heaviest precipitate was found in endothelium of vessels, banal inflammatory cells, and fibroblasts.

(7) *Multiple Idiopathic Hemorrhagic Sarcoma.*—Multiple Idiopathic Hemorrhagic Sarcoma of Kaposi (Six Specimens): A wide range of pathologic, morphologic, and enzymatic activity was noted in lesions of Kaposi's sarcoma, and therefore the specimens will be reported upon individually.

Specimen 1: Early stage. With hematoxylin and eosin increased vascularity was seen throughout the cutis. In the depths dilated blood spaces were seen, separated by spindle cells and lymphocytic infiltrations. There was marked activity in the smaller, more superficial vessels, whereas the deeper, dilated vessels showed little or no activity. Nuclei of lymphocytes and spindle cells showed varying degrees of positivity.

Specimen 2: Early stage. Hematoxylin and eosin showed moderate increase in vascularity and lymphocytic infiltration but no substantial spindle-cell proliferation. Activity was demonstrated in most, but not all, of the newly formed vessels, and mild deposit was seen on the collagen fibers between them.

Specimen 3: Early stage. With hematoxylin and eosin there was substantial vascular proliferation, surrounded by heavy lymphocytic infiltration, and some spindle-cell proliferation in the upper half of the cutis. Almost all the vascular linings showed moderate activity. There was milder activity in the proliferating intervascular spindle cells and in relation to the lymphocytic infiltrates.

Specimen 4: Moderately advanced stage. With hematoxylin and eosin the larger part of the cutis was occupied by collections of engorged, dilated thin-walled blood spaces, between which were seen strands of spindle cells admixed with lymphocytes. To one side of the zone so affected, and resembling the more typical Kaposis sarcoma, tissue was seen with poorly formed vessels, surrounded by heavy lymphocytic cuffs, and bands of spindle cells. Most of the vessels with "normal" lumens showed endothelial activity, in striking contrast to the groups of dilated blood spaces, the linings of which showed no deposit. However, in the stroma between the wide channels there were AP-positive blood vessels. The intervascular spindle cells showed mild activity.

Specimen 5: Moderately advanced stage. Hematoxylin and eosin showed increase in the number of vessels throughout the upper cutis, many of which were poorly formed and had incomplete endothelial linings. Around and between them there was spindle-cell proliferation and lymphocytic infiltration. Alkaline phosphatase stains showed numerous vessels with activity, although many vascular channels, some well-formed with thick walls as well as poorly formed ones, showed no activity. The areas of spindle-cell proliferation, particularly in the upper cutis, showed considerable activity. Milder deposit was found in the lymphocytic nuclei and in the subcutaneous fat trabeculae.

Specimen 6: Early sarcomatous stage. Hematoxylin and eosin showed loss of epidermis, granulation tissue with numerous newly formed blood vessels in the upper cutis, and, in the deeper cutis, massive spindle-cell hyperplasia with plasmocytic and foreign body type giant-cell infiltrate. There was a great increase in number of highly AP-active vessels in the upper cutis. The bundles of spindle cells demonstrated strong AP positivity (Fig. 19), more concentrated toward the periphery of these cells.

Giant cells were AP-negative and thereby stood out from the dark background.

Summary: Kaposi's sarcoma in the various stages of progression (early, moderately advanced, sarcomatous) manifested differences in AP activity as follows:

(*a*) Smaller vessels, both those of normal appearance and those poorly formed, generally showed moderate AP activity.

(*b*) Larger and dilated blood spaces often showed no activity.

(*c*) Intervascular spindle-cell proliferations showed varying measures of activity, which were apparently not correlated with the stages (early vs. late) of such proliferations. The sarcomatous stage showed marked AP activity of spindle cells.

(*d*) The inflammatory (mainly lymphocytic) infiltrates showed activity mildly or not at all, and, when present, it was mostly nuclear. In one specimen with foreign body giant cells these were devoid of deposit.

(8) *Benign Tumors and Hamartomas.*—(*a*) Epidermal: Intraepidermal nevus and seborrheic keratosis (12 specimens). Alkaline phosphatase findings in this group showed no significant changes in relation to the hyperplastic epidermal cells. Hyperkeratotic stratum corneum often showed false positive precipitate. There was an increase in the papillary capillaries, which were highly active, so that they stood out from the background of banal inflammatory cells showing little or no activity. One specimen had mild activity of the subcutaneous fat trabeculae.

Cutaneous horn (one specimen). The keratin showed mild, false positive precipitate distributed in a laminated fashion, appearing to fill the minute clefts between the cornified cells. The stratum mucosum showed some changes like those in Bowen's disease. It contained no cobalt sulfide, except for the nucleoli of cells overlying centers of high AP activity in the papillary capillaries. Strongly positive capillaries were increased in number subjacent to the horn. There was a considerable inflammatory infiltrate high in the cutis which showed no activity. The elastica was basophilic and showed mild false positive precipitate.

Acanthoma (one specimen). In acanthoma the uppermost cutis showed moderate AP activity in relation to newly formed connective tissue, and many new highly active vessels were seen. There was a mild precipitate (principally nuclear) in inflammatory cells. The subcutaneous fat trabeculae showed heavy AP activity.

Keratoacanthoma (two specimens). There was an increase of AP-positive vessels around the acanthotic proliferations. In contrast to most prickle-cell epitheliomas, the stroma showed little or no activity of the fibrillar inflammatory cells.

(*b*) Adnexal: Epithelial cyst (three specimens). There was false positive precipitate in the keratin contents of the cysts, which was progressively heavier from the periphery toward the center. This precipitate was more granular than cobalt sulfide deposit representing AP activity and was most pronounced lining the spaces between the stratified cells.

Calcifying epithelioma (one specimen). AP activity was limited to connective tissue fibrils, inflammatory cells, and increased vessels surrounding the tumor. The tumor itself showed no activity. Near the center of the tumor lobules deposits of calcium produced false positive precipitation.

Follicular cyst (one specimen). The keratin was arranged within the cyst in concentric layers, resembling an onion in cross section. There was a false positive precipitate correspondingly laminated. The epithelial wall showed no AP activity. There was an increase of capillaries with AP activity surrounding the cyst and some deposit in nuclei of the banal infiltrate and on connective tissue fibers.

Trichoepithelioma (16 specimens, all from different sites obtained from the same patient). Apart from the cysts, islets of "basal" cells, and prolongations of similar cells arising from the cyst walls, there was heavy inflammatory infiltrate (often of foreign body type), vascular proliferation, and fibroblastic reaction.

At the tips of many islets and their prolongations there were crescentic areas of intense AP activity, which were interpreted as representing hair papillae (Fig. 9).

The stroma in some areas showed strong AP activity of fibroblasts. The vascular endothelium showed substantial activity, as did some of the nearby inflammatory cells, with the specific exception of the foreign body type giant cells, which were invariably inactive.

Senile sebaceous hyperplasia (two specimens). One specimen of senile sebaceous hyperplasia showed increased vascularity throughout the cutis and a false positive precipitate on the degenerated elastica. Although the sebaceous glands were hyperplastic, no activity of the acini was discernible. In addition, the second specimen had substantial deposit in fibrillar cells of the stroma and in relation to the inflammatory infiltrate, where it was mainly nuclear.

Rhinophyma (two specimens). With hematoxylin and eosin the outstanding features were intense fibroblastic reaction, edema, vascular dilatation, and heavy inflammatory reaction. Neither specimen showed hyperplasia of sebaceous glands. Alkaline phosphatase activity was seen in relation to fibroblasts, endothelium of the dilated vessels, and some inflammatory cells, particularly those near positive vessels.

Adenoma sebaceum (one specimen). AP activity was seen in those elements normally positive in skin, but which in this material were present in greater numbers, i. e., follicles and vessels.

Sebaceous adenoma (one specimen). The specimen showed a conglomeration of lobules. In comparison with normal, the germinative cells were many layers thick, deeply basophilic, and less flattened. These germinative cells of the adenoma lobules showed heavy nuclear and cytoplasmic deposit (Fig. 7); in relation both to normal and adenomatous glands the perisebaceous capillary networks were AP-active.

Syringoma (three specimens). None of the cells lining the cysts showed AP activity. Some cysts showed a false positive precipitate in their lumens.

Hydrocystoma (one specimen). The specimen showed dilated cystic spaces lined by one or more layers of epithelium. There was moderate AP activity of the epithelium, more noticeable in the cells lining the lumens and more particularly at their free edges (Fig. 13). Within the lumens there was amorphous material showing considerable deposit (controls were not carried out to determine whether this was true or false positivity).

Mucous retention cyst (one specimen). The adjacent normal mucous glands showed mild AP activity of the peripheral acinar cells, most marked in the nucleoli. The cyst mass (and surrounding capsule) showed heavy fibrillar deposits. The amorphous contents of the cyst showed mild activity of nuclear and cellular debris.

(*c*) Mesodermal: Achrochordon (three specimens). The papillary capillaries were few in number but normal in size, shape, and AP activity. In the center of the growths a few inactive dilated vascular spaces were seen.

Fibroepithelial papilloma (mucous membrane) (one specimen). Unlike the achrochordons, the mucosal papilloma showed intense AP activity in the fibrillar stroma and in the numerous vessels which made up its "stalk." The epithelium covering the specimen showed no deposit.

Cutaneous nodule (15 specimens). With hematoxylin and eosin two main types were distinguishable. The first, or cellular, variety consisted of short spindle, triangular, or irregular cells (which will be designated Type I cells). These cells were compact, so that in any given field numerous nuclei were seen (Fig. 16). The second variety was composed of long spindle cells (Type II cells), and in any given field there were relatively few nuclei (Fig. 16). These long spindle cells were, in some cases, apparently laying down collagen. Five specimens were of the Type I and three specimens of Type II cells. Four specimens showed both varieties, with Type I cells usually being sit-

uated toward the periphery of the tumors and more frequently in the upper cutis. Three specimens showed intermediary cellular structure; they could not be classified as Type I or Type II. These cellular types were found to be independent of other characteristics such as lipid or hemosiderin deposits or vascular components.

The distribution of AP was found to be strikingly different in the two cellular types (Fig. 17). In Type I lesions the enzyme was limited to capillaries (nucleus and cytoplasm of endothelium) coursing throughout the tumor. No cobalt sulfide was seen in the fibrocellular components of the specimens. Cutaneous nodules composed of Type II cells, in addition to having AP activity in capillary endothelium, showed variable amounts of precipitate on the fibroblasts themselves (Fig. 18), in their cytoplasm as well as on the nuclei. At times the AP seemed to line spaces between the cells, resulting in a lace-like arrangement.

In the combined variety, AP activity was limited to capillaries in areas composed of Type I cells, while in areas of Type II cells the deposit was seen in fibroblasts as well as in endothelium (Fig. 17).

The intermediary cellular types showed varying pictures, with AP activity in capillaries and, at times, on long fibrocytes.

Some cutaneous nodules showed cobalt sulfide in the fat trabeculae, usually more pronounced in the area immediately related to the tumor.

Keloid (one specimen). Hematoxylin and eosin revealed a "fresh" keloid in the middle two-fourths of the cutis, composed of bundles of light-staining vacuolated spindle cells, between which there were long thin-walled capillaries. The spindle cells and capillaries showed considerable AP activity. The deposit in the fibroblasts was principally on the nuclei and apparently lining the periphery of the cells.

Scar tissue (one specimen). Hematoxylin and eosin revealed fibroblastic proliferation with an infiltrate of banal inflammatory and foreign body giant cells. There was heavy AP activity, mainly on the proliferating fibrillar cells and the inflammatory infiltrate. However, the foreign body giant cells stood out clearly as containing very little or no deposit.

Lipoma (four specimens). Lipomas were studied because AP was found, though inconsistently, in the subcutaneous tissue of normal as well as pathologic material. All specimens showed mild deposit on the fat trabeculae but none in the empty spaces resulting from processing. The vessels seen in these trabeculae always showed only mild activity, in contrast to the generally high activity of papillary capillaries.

Neurofibroma (von Recklinghausen's disease) (two specimens). Both specimens were devoid of AP activity, except for that demonstrated in the sparse vessels coursing throughout the tumor. The neurofibroma cells themselves showed no deposit.

(*d*) Cellular Nevi: Intradermal nevus (21 specimens). With two outstanding exceptions, nevus cells themselves were devoid of activity. Moderate AP activity was seen in relation to increased vascularity, while in inflammatory cells there was mild to no deposit.

In the two exceptions mentioned, groups of nevus cells in the midcutis showed moderate AP activity. One of the specimens showed a fibroblastic reaction, with moderate activity around the nevus cells islets. Groups of nevus cells, some near the fibroblastic reaction, others more remote, showed moderate deposit in their nuclei and cytoplasm. In the other specimen there was intense AP activity limited to groups of nevus cells in one area of the lesion (Fig. 15). The precipitate was so dense that the outlines of the cells could not be recognized. In addition, many capillaries and a few inflammatory cells with AP activity were seen around them.

One specimen was from a lesion which had been treated eight years previously by desiccation and curettage. The cutis showed widespread homogenization, diminishing toward the depth. Throughout the affected connective tissue there coursed large thin-walled blood vessels, the linings of which

were devoid of AP activity. In contrast, normal appearing capillaries in unaffected areas showed moderate activity.

Compound nevus (one specimen). Neither junctional nor intradermal elements were associated with AP activity.

Blue nevus (one specimen). Activity was limited to vessels in the upper cutis, which were only slightly increased in number in relation to the area of the blue nevus cells. There was no deposit on the nevus cells.

(9) *Precancroses.*—Bowen's Disease (One Specimen): In the cutis underlying the typical areas of Bowen's disease, there was activity mainly in the walls of the increased capillaries but there was little or no activity in the cellular infiltrate surrounding these vessels.

(10) *Malignant Tumors.*—(*a*) Epitheliomas: Basal-cell epithelioma (36 specimens). In all specimens proliferation of highly AP active capillaries was seen surrounding the epithelioma groups. Eight specimens showed deposit in the inflammatory infiltrates, which was usually more pronounced in the nuclei and even more so in the nucleoli.

Sixteen specimens showed mild to pronounced activity of the fibroblastic stroma near the epithelioma masses. The deposit was heaviest in those specimens in which the fibroblastic response was in the early stage, and, contrariwise, mild or absent where the stroma was more "mature," as judged by its tendency toward laying down collagen.

Eleven specimens showed mild to moderate degrees of AP activity in the tumor cells themselves. Generally speaking, this activity was more intense in the cell groups situated nearer the periphery of the tumor, particularly in the palisade or outermost layer of the epithelioma islets. Activity was seen in cystic, adenoid, "en masse," and meta-typical varieties of epithelioma. The crusts overlying ulcerated lesions often showed diffuse (false positive) deposits.

One specimen bore some resemblance to trichoepithelioma with hematoxylin and eosin. It was removed from the cheek of a 28-year-old white man, according to whom the lesion was of 10 years' duration. The sections showed mild AP activity at the periphery of some of the epithelioma groups. The outlines of some islets gave the appearance of invaginating rudimentary hair papillae, but the stroma of these "papillae" showed no AP activity. The lack of deposit here was in contrast to the "papillae" of trichoepithelioma, which showed intense precipitates.

Fat trabeculae frequently showed mild activity limited to the areas directly beneath the epitheliomas.

False positive precipitates were frequently seen in the contents of the "cysts" of basal-cell epitheliomas showing central breakdown. Similar false positive deposits were found on the degenerated elastica of senile skin or of exposed areas.

Multicentric superficial basal-cell epithelioma (one specimen). The epithelioma buds themselves showed no AP activity. There was a general increased vascularity of the upper cutis with mild activity, and there was light deposit on the connective tissue fibers adjacent to the epithelioma.

Prickle-cell epithelioma (nine specimens). Hyperkeratosis, when present, as well as horny pearls, showed false positive deposits. The tumor cells themselves showed no AP activity. The outstanding site of enzymatic action was the upper cutis, subjacent to the proliferating prickle cells. Here there was a variety of pictures—from simple vascular hyperplasia (with active endothelium) to intense activity of proliferating connective tissue fibrils and inflammatory cells. There appeared to be greater activity in the connective tissue adjacent to the well-differentiated neoplasms than in the more undifferentiated tumors. The subcutis underlying tumor proliferations often showed moderate AP activity.

(*b*) Malignant melanoma (one specimen). The specimen showed large well-defined cell groups. There were few junctional elements. Some cells with atypicalities, mitoses, pleomorphism, and intense melanin pigmentation were present. The tumor cells themselves were practically free of AP activity, with the exception of mild precipitate

on the nucleoli and minimal staining of the nuclei. Moderate AP activity was seen in the connective tissue and inflammatory cells in the upper cutis adjacent to the tumor. In the corium deep to the lesion deposit was seen lining the surfaces of mature collagen fibers.

(11) *Lymphomas.*—Lymphocytoma Cutis (one specimen): Hematoxylin and eosin demonstrated well-defined masses of lymphogenous elements in the cutis with germinal center-like architecture. The central areas of these conglomerations showed few vessels, and these had AP active endothelium. However, towards the periphery of these cell groups there was a great increase in vascularity, with strongly positive endothelium and substantial deposit in the small round cells adjacent to these vessels.

Mycosis Fungoides (two specimens): Both specimens showed increase of vessels in the upper and midcutis, surrounded by polymorphous infiltrates. All the vessels showed moderate to marked AP activity. The infiltrative cells showed varying measures of activity, especially of their nuclei, those cells lying closest to the vessels showing greatest activity. Cells within the Pautrier's microabscesses in both cases were devoid of activity.

Lymphoma—Type Undetermined (Three Specimens): All specimens showed increased vascularity, and in two the lumens were dilated. All vessels showed moderate AP activity. The vessels were surrounded by infiltrates, composed of small round cells, histiocytes, and abnormal cells showing pleomorphism, mitoses, giant nuclei, etc. These infiltrates showed mild to moderate activity when situated near active vessels. One specimen showed a rather dense perivascular fibrillar activity, apparently related to proliferating connective tissue fibers.

Leukemia, Unclassified (One Specimen): Activity was limited almost entirely to numerous fine capillaries in the upper cutis and midcutis. The leukemic infiltrate surrounded these vessels. In only a few areas was mild nuclear activity seen in the leukemic cells, and this was apparently due to diffusion from nearby vessels with high activity. In general, the infiltrating cells lacked activity.

IV. Comment

A. Alkaline Phosphatase Distribution in Normal Human Skin

The findings described in this study are in general agreement with those reported by other observers, however, with certain exceptions.

On comparison with control sections, granules in the stratum granulosum showed false positive cobalt sulfide precipitates but no AP activity. True phosphatase activity in these structures was reported by Pirilä and Eränkö.[156]

The acinar cells of sebaceous glands generally showed no activity, and activity when present was seen only as faint postivity in the most peripheral cells of the lobules (in these, mostly nuclear and, more particularly, nucleolar). The results of Pirilä and Eränkö [156] are in agreement with these findings, whereas Montagna [135] reported these glands to have acinar activity. This apparent discrepancy may be explained by the fact that the sebaceous glands studied by Montagna were those of the external auditory canal, which were not investigated in this study.

B. Alkaline Phosphatase Activity in Diseases of the Skin

From the material thus far accumulated certain facts concerning AP distribution in skin disease are here summarized.

1. Almost all disease associated with blood vessel proliferation (hemangiomas, granulomas, inflammatory dermatoses, angiosarcomas, etc.) show substantial over-all increase of AP activity in relation to newly formed vessels. This increase appears in the early stages of these diseases, when the vessels are still "immature." As the lesions progress in age, some, but usually not all, vessels lose their endothelial activity and become AP-negative. Vessels which have undergone dilatation frequently show reduced or no activity.

The endothelium of the lymphatics, in the one lymphangioma studied, showed no activity.

2. Those diseases associated with inflammatory, rather than vascular, pathology (e. g., drug eruption, lichen planus) generally show little AP activity in relation to inflammatory cells. Some exceptions exist, and when activity is seen it apparently represents (at least in part) diffusion of the enzyme, its activators, or its products of hydrolysis rather than true in vivo distribution.

On the other hand, those diseases with inflammatory infiltrates associated with vascular hyperplasia and fibroblastic reaction frequently show intense AP activity of the inflammatory cells (here either representing the in vivo situation or increased diffusion). Acute banal inflammatory conditions usually show greater activity than chronic ones. Pure epithelioid and reticuloendothelial proliferations (e. g., sarcoid, central portions of tubercles, giant cells) generally show no AP activity.

3. Dermatoses associated with proliferation of fibroblasts show variable AP activity in these elements. Normal connective tissue has no activity. Early stages of connective tissue proliferation (e. g., keloid, scar tissue, cutaneous nodules, etc.) usually have moderate to intense AP activity. These conditions in later stages (i. e., in which the connective tissue is more mature and organized) and benign neoplastic proliferation (e. g., neurofibroma) show little or no AP activity.

It was frequently noted that AP appears to line the surface of the collagen fibers in those conditions showing connective tissue proliferation. In Rothman's text,[168] Wells points out that there is increase metachromasia and periodic acid-Schiff-positive material (mucopolysaccharide) of the ground substance at sites of early fibroblastic formation. It is apparent that the distributions of alkaline phosphatase and mucopolysaccharides are similar, and a possible relationship between them is suggested (Moog [137]).

4. Studies of two diseases of the follicular apparatus led to interesting observations. The first, trichoepithelioma, shows numerous hair papillae irregularly distributed at the periphery of the "epithelioma" masses, producing a striking picture of intense AP activity (Fig. 10).

The second condition in this group is alopecia areata. Hair papillae in specimens of normal skin almost always show intense AP activity. Serially sectioned specimens of alopecia areata show many AP-inactive papillae at the onset of the hair loss. Since inactivity has been reported in animals in the catagen stage of their hair cycles, it is not surprising that similar findings prevail in the early stages of hair loss in alopecia areata. However, the observation is of interest and suggests a basic metabolic defect in this disease.

5. Study of AP activity in benign and malignant tumors of the skin resulted in the following findings:

(*a*) Benign neoplasms: (1) Adnexal tumors. The peripheral portion of the lobules in sebaceous adenoma show marked AP activity.

Cells lining the cystic spaces of hydrocystoma are moderately active.

The lining of the cysts of syringoma show no AP activity.

Trichoepithelioma shows active hair papillae.

These findings may be explained in the following manner. The activity of sebaceous adenoma in contrast to normal sebaceous glands (which have minimal or no activity) may be due to increased phosphatase present in early histodifferentiation of this tissue. In this regard, it has been repeatedly demonstrated that most structures show AP activity in their embryonal stages, even though the fully developed tissues have no demonstrable enzyme (Moog [137]).

Normal sweat glands are active, whereas the ducts show no activity. It is therefore suggested that syringomas, being AP-negative, are possibly of ductal origin. Although hydrocystomas are AP-positive, their acinar origin is doubtful.

The AP activity of "papillae" seen in trichoepitheliomas speaks strongly in favor of these lesions being hamartomas of follicular apparatus.

(2) Junction, intradermal, and combined nevi. In general, the entire group of benign cellular nevi shows no AP activity of the nevus cells. Only two exceptions were observed. Both were intradermal nevi in which some, but not all, of the nevus cell groups showed mild activity. It is possible that these islets may have been composed of cells of recent formation.

(3) Benign reticuloendothelial proliferations. Lymphocytoma cutis shows no AP activity of the avascular "germinal center-like" areas, but moderate activity of the surrounding vascularized tissue infiltrated with lymphocytes.

The cellular elements in a specimen of "facial granuloma" show no AP activity, other than in some of the multinucleated giant cells.

(4) Lipoma. Mild trabecular AP activity was seen in all the specimens of lipoma studied. This is in contrast to generally inactive normal adipose tissue.

(*b*) Malignant neoplasms: (1) Epithelioma. a. Prickle-cell epithelioma. No specimens of prickle-cell epithelioma showed AP activity in relation to the tumor cells themselves.

b. Basal-cell epithelioma. Almost one-third of the basal-cell epitheliomas show AP activity of the tumor cells. It is not possible to predict which histologic type of basal-cell epithelioma will show phosphatase activity and which will not.

(2) Sarcoma. The only specimen of sarcoma studied was an advanced lesion of Kaposi's disease. This specimen shows intense AP activity in the sarcomatous elements.

(3) The specimen of leukemia showed little or no activity in the infiltrative cells. The group of malignant lymphomas (mycosis fungoides and certain unclassified lymphomas) show a rather consistent AP activity in relation to the neoplastic infiltrates. However, it should be noted that the activity is invariably most intense in those cells nearest highly active capillaries, and this suggests that some of the enzyme demonstrated in the cells is produced by diffusion from these capillaries and does not actually represent the true in vivo AP distribution.

(4) Malignant melanoma. The specimen available for study of nevocarcinoma shows no AP activity in the melanoma cells themselves.

(5) Stromal reaction in relation to malignant neoplasms. Moderate to intense AP activity is frequently observed in stromal response to invading tumors. The active stroma, composed of proliferating capillaries, fibroblasts, and inflammatory cells seems to behave as a purposeful barrier to the advancement of the neoplasm, rather than merely acting as the irritative response to a foreign body. The intense AP activity associated with this barrier speaks for the importance of AP in these reactions.

(*c*) From these findings a few broad inferences can be drawn. Alkaline phosphatase is an enzyme which is intimately associated with many different structures and functions of the skin.

In normal skin it is principally found at sites of high metabolic activity (e. g., capillary walls, hair papillae, sweat glands), and it is not seen in sites of low metabolic activity (e. g., mature collagen, adipose tissue). This is also apparently true of pathologic processes of the skin. Therefore, diseases manifesting active proliferation (e. g., granulation tissue, acute inflammatory infiltrates) are frequently found to have increased AP activity. Moreover, proliferating elements of tissues which in normal skin contain no significant quantities of enzyme show measures of activity in pathologic lesions (e. g., the AP activity seen in sebaceous adenoma and lipoma). Nevertheless, this comparison has its limitations in that some actively proliferating elements show no increased AP activity (e. g., prickle-cell epithelioma, epithelioid hyperplasias etc.). The epidermis, even during periods of active proliferation (and pre-

sumably high metabolic activity), shows little or no phosphatase.

V. Summary and Conclusions

Alkaline phosphatase is an enzyme which has the capacity to liberate phosphate from a variety of phosphate-containing compounds. The nature and physiologic significance of this enzyme are outlined.

Alkaline phosphatase was demonstrated in 29 normal and 224 pathologic specimens of human skin, with use of the Gomori-Takamatsu technique.

Distributions of alkaline phosphatase in normal and pathologic skin, as previously reported, are tabulated.

The optimum incubation period for skin specimens with use of the Gomori-Takamatsu method was found to be four hours.

Control sections were studied where indicated. False positive findings were found in the following elements: soft keratin, inner root sheath of the hair follicle, hair shaft medulla, keratohyalin granules, degenerated elastic tissue, smooth muscle, "colloid," and sites of preformed calcium.

The distribution of alkaline phosphatase in specimens of normal human skin from various areas is described. The relative phosphatase activities of various components of the skin are as follows:

(*a*) Marked alkaline phosphatase activity: hair papillae

(*b*) Moderate alkaline phosphatase activity: capillaries, eccrine sweat glands, external connective tissue sheath of the hair follicles

(*c*) Mild alkaline phosphatase activity: hair bulb matrix cells, apocrine sweat glands

(*d*) Weak alkaline phosphatase activity: subcutaneous fat trabeculae (some specimens)

(*e*) No demonstrable alkaline phosphatase activity: all epidermal strata, collagen and elastica, all elements of the follicular apparatus (with the exception of those mentioned above), eccrine and apocrine ducts, sebaceous glands (with rare exceptions of weak activity in the peripheral cells), nerves, smooth muscle.

The distribution of alkaline phosphatase in diseases of the human skin is reported upon. The outstanding findings are the following:

(*a*) Early stages of vascular proliferation (e. g., hemangiomas, granulomas, etc.) show marked alkaline phosphatase activity, later stages show reduced or no activity.

(*b*) Banal cellular infiltrates associated with acute inflammatory dermatoses often display moderate to marked activity, whereas the cellular components of chronic inflammatory conditions frequently show little or no activity. Epithelioid cellular infiltrates are generally AP-inactive.

(*c*) Early phases of connective tissue proliferation (e. g., cutaneous nodule, keloid) are associated with moderate to marked degrees of alkaline phosphatase activity, whereas late stages show little or no activity.

(*d*) Benign neoplasms frequently show an activity similar to that of the tissue from which they originated (e. g., trichoepithelioma, neurofibroma); exceptions occur (e. g., sebaceous adenoma, some intradermal nevi).

(*e*) Malignant neoplasms show variable alkaline phosphatase activity. Prickle-cell epithelioma and malignant melanoma show no phosphatase activity of the malignant cells themselves. In contrast, about 30% of basal-cell epitheliomas show alkaline phosphatase activity in relation to the tumor cells. Kaposi's sarcoma shows intense phosphatase activity.

In general, alkaline phosphatase is demonstrable at sites of high metabolic activity, both in normal and diseased skin. However, the precise role that this enzyme plays in cutaneous metabolic processes remains obscure.

Dr. Francisco Kerdel Vegas, of Caracas, Venezuela, gave assistance in preparing the slides and collecting the material. Dr. Sonia Dobkevitch-Morrill gave freely of her time in planning the experiments and controls and reviewing many of the sections. The photography was performed by Mrs. Eleanor Moreland. Mrs. Dorothy Poli, Miss Shirley Simms, Miss Judith Kallman, and Miss Nanett Silver gave technical and clerical help.

New York University Post-Graduate Medical School.

ABSTRACT OF DISCUSSION

Dr. Stephen Rothman, Chicago: Dr. Kopf has just quoted Dr. Gomori, the leading expert in this field, as stating that nobody has any idea about the role and significance of the alkaline phosphatase and its localization in certain tissue elements.

It is quite natural and justified to ask whether it is worth while to deal with a phenomenon, the biological significance of which is unknown or possibly nonexistent. The answer to this question is definitely "yes." Many an originally esoteric, highly impractical, and apparently meaningless finding, with no interpretation at hand, has proved later on to be of great biological significance.

On close scrutiny, we find, as indicated by Dr. Kopf, that alkaline phosphatase is not an absolute *terra incognita*. We understand a few things about it. Bone formation depends on the product of calcium ion concentration times phosphate concentration. Alkaline phosphatase, being present in bone tissue and being able to split off phosphate ions from organic radicals, may increase this product and thus promote bone deposition. In all conditions in which there is formation of bone, either in repair processes or neoplastic conditions, the alkaline phosphatase content of the blood is increased, and this finding has by now great clinical significance.

In spite of our ignorance, not knowing what alkaline phosphatase is doing in the skin, the findings of Dr. Kopf have been most valuable. He has found a definite relationship between the presence of this enzyme and lively cellular proliferation, even in cell types where the same cell, in a mature, less lively proliferating state, does not show enzymatic activity. This, he has shown, is true for angiomas, granuloma pyogenicum, and sebaceous adenomas. This finding may have quite considerable practical significance. He has given some differential diagnostic points in the distinction of trichoepitheliomas and basal-cell carcinomas. He was able to distinguish two clearly different cell types in subepidermal fibrosis, and he confirmed older findings concerning the striking differences in the alkaline phosphatase content of eccrine and apocrine glands.

Earlier, Spier and Martin demonstrated the strong positivity of the active hair papilla, a finding now confirmed by Dr. Kopf. Interestingly, these German authors have pointed out that the acid phosphatase is positive, even in the ectopic sebaceous glands of Fordyce's disease, although they are not supposed to function at all—a finding quite analogous to Dr. Kopf's finding in trichoepitheliomas.

I happen to be an old friend of Dr. Gomori, who is the father of the histochemical phosphatase reactions. We worked in the same department. As I am not an expert in histochemistry, I took the liberty to show him Dr. Kopf's manuscript and illustrations. He found that Dr. Kopf used a correct technique, applied the right controls, and distinguished with accuracy the true and false positive reactions.

I wish to congratulate Dr. Kopf on his fine work and on the great honor bestowed upon him by the American Dermatological Association.

Dr. Walter F. Lever, Boston: Could this method not be used in a study of the possible role of myoepithelial cells in various tumors? Dr. Kopf showed that in both eccrine and apocrine sweat glands the myoepithelial cells have a strongly positive activity. He also showed that the peripheral cells in basal-cell epithelioma have a positive activity.

I would surmise, although cautiously, that, perhaps, the peripheral cells of basal-cell epitheliomas are differentiating toward myoepithelial cells. Has Dr. Kopf carried out any studies of cylindroma? In cylindroma one sees a differentiation into two types of cells much better than in basal epithelioma, in that there is one type of cell with a small dark nucleus and another cell type with a large pale nucleus. I have always suspected that the cells with small dark nuclei may be differentiating toward myoepithelial cells.

I should also like to know if Dr. Kopf has carried out any studies on the so-called spiradenoma, which was described before this Association one year ago by Dr. Kersten and which I have described before (or, at least, a tumor very closely related to it), under the name of myoepithelioma (*Arch. Dermat. & Syph.* 57:332, 1948), assuming that many of the spindle cells in this tumor were differentiating toward myoepithelial cells.

Dr. George C. Andrews, New York: It has long been suggested that the action of irradiation on the skin is due to some effect upon the enzymes in the skin. I should like to ask Dr. Kopf if he has made any studies, perhaps, of skin before and after irradiation. I would also, in this connection, be interested in the hemangiomas. The work with retrolental fibroplasia has pretty definitely proved that it is caused by hyperoxygenation due to giving oxygen to the babies, and the disease has practically disappeared since that practice has been stopped. Of course, with the retrolental fibroplasias, there are often associated hemangiomas, and, since the alkaline phosphatase has been shown to be present in hemangiomas, I should be interested to know if, perhaps, studies should not be made of retrolental fibroplasia for alkaline phosphatase and, also, whether we might find alkaline phosphatase in nevus flammeus, which does not respond to irradiation.

Dr. Frank C. Combes, New York: This subject certainly opens up a wide field for investiga-

tion. The application of this method of determining alkaline phosphatase activity may explain the modus operandi of many types of therapy which are now rather obscure—that is, much of our so-called empiric therapy.

I am sure the idea came to the minds of many of you as, I imagine, it may have done to Dr. Kopf, that in alopecia areata the absence of alkaline phosphatase activity in the hair papilla may be restored by steroid therapy. I was wondering whether he had ever given any of these patients cortisone or steroid hormones, and what effect they might have on the hair papilla. Is there a return of alkaline phosphatase activity with this regrowth of hair?

I would also be interested in having him continue work on the response of many dermatoses to vitamin A therapy. We know that vitamin A itself does not exist in the skin. Some dermatoses do respond to it, but not with any regularity. Keratosis follicularis does, and some cases of leukoplakia. I was wondering whether there was a change in alkaline phosphatase activity which would explain this.

I am also interested to know the effects of radiant energy of various types on alkaline phosphatase activity.

Dr. Everett C. Fox, Dallas, Texas: Dr. Kopf stated that nevus cells showed varying activities of alkaline phosphatase reaction, that malignant melanoma showed no activity. I should like to ask him if he studied enough slides, or if it is worth while continuing that study, and if there might be a possibility at times of nevus cells with activity that are malignant. The alkaline phosphatase reaction may offer a very interesting approach to studies in the differentiation of malignant melanoma.

Dr. Louis H. Winer, Beverly Hills, Calif.: Dr. Kopf demonstrated to us this morning that in nodular dermatofibrosis there is increased alkaline phosphatase in the cutis where the proliferating cells engulf the normal connective tissue, either cannibalizing or trapping the normal connective tissue within the proliferating long fibrotic bundles.

The same high alkaline phosphatase reaction was observed in carcinoma, where mainly a degeneration of collagen was observed. Degenerated collagen underneath a carcinoma of the prickle-cell type has been assumed to be a precursor of the carcinomatous process. I should like to ask Dr. Kopf, "Is it the collagen degeneration that gives this increased phosphatase reaction?"

Dr. Alfred W. Kopf: I should like to thank the discussers for their interesting comments and helpful suggestions.

I thank Dr. Rothman for acquainting me with the work of Spier and Martin in which they reported acid phosphatase in ectopic sebaceous glands of Fordyce's condition.

One comes to the conclusion in working with alkaline phosphatase that this enzyme is in some way required for growth and histodifferentiation of all structures. Dr. Moog has studied this aspect in embryonic development in animals and found that all tissues at some stage of development contain alkaline phosphatase which is demonstrable histochemically. In these embryos, even the epidermal strata and dermal connective tissue components had associated phosphatase activity. We had occasion to study skin biopsy specimens from three premature infants, and, interestingly enough, the connective tissue, which in normal adult skin has no demonstrable alkaline phosphatase, showed enzyme activity. Apparently it is an enzyme needed for growth and metabolic function in general, although the exact substrates upon which it acts in the skin are unknown at present.

Dr. Winer's point was very well taken. He asked whether alkaline phosphatase activity would be "produced" at the sites of degeneration of collagen surrounding areas of invasion by basal-cell epitheliomas or in cutaneous nodules. This thought has occurred to me, and there are certain diseases, such as granuloma annulare, in which this association has been demonstrated.

I think Dr. Andrews remarks were most interesting and thought-provoking. It has been previously shown that not only chemical but also physical agents can inactivate alkaline phosphatase. Both ultraviolet light and x-rays have been shown to inactivate this enzyme. It is for this reason that I have contemplated doing further studies along these lines. I hope to find out what happens to alkaline phosphatase in patients with tinea capitis who receive epilating doses of x-ray. Also, I have considered irradiating biopsy specimens to study the effect of ionizing radiation on the enzyme's activity.

I am not sure if the distribution of alkaline phosphatase has been investigated in retrolental fibroplasia, but I think it would be very interesting to do so. I suspect that alkaline phosphatase would be demonstrable histochemically in the proliferating elements of the tissues. In reference to nevus flammeus, I had no case for study, but I should like to find out if the vessels composing this variety of hemangioma have the same associated intense activity shown by most of the strawberry hemangiomas I reviewed.

I cannot give a direct answer concerning Dr. Lever's point about myoepithelial cells showing alkaline phosphatase activity and whether this finding could aid in deciding if the cells of a given basal-cell epithelioma originated from primary epithelial germ or myoepithelial cells. I had no specimens of cylindroma or spiradenoma for study. However, I did have several specimens of syringoma, which invariably showed no phosphatase activity. On the other hand, hydrocystoma showed

activity. On the basis of these findings one might theorize that syringomas are of sweat duct origin, since, like sweat ducts, they are inactive, whereas hydrocystomas are of sweat gland origin, since acinar and myoepithelial cells of sudoriferous glands are associated with enzyme activity.

Dr. Combes' question regarding the effect of hydrocortisone on the papillae in alopecia areata is most interesting. Dr. Orentreich, of the New York Skin and Cancer Unit, has injected hydrocortisone acetate as well as some of the newer delta analogues into hairless areas of alopecia areata, with resulting temporary regrowth at the sites of administration. We have had the opportunity of performing biopsies of several such areas and have found alkaline phosphatase activity in the growing hair papillae in these areas. I cannot answer your inquiry, Dr. Combes, about the effect of vitamin A on skin alkaline phosphatase activity, since I do not recall any patients in our series with avitaminosis A or any receiving vitamin A therapy.

I would like to comment briefly on Dr. Fox's point. What does it mean that the nevus cells in only 2 of 21 specimens of dermal cellular nevi showed intense alkaline phosphatase activity? It may possibly mean that these cells are in a "growing" or "differentiating" stage. Since we had only one specimen of malignant melanoma, it would be unwarranted to surmise that all specimens of this condition would show absence of histochemically demonstrable alkaline phosphatase.

REFERENCES

1. Akamatsu, S., and Kobayashi, K.: Co-phosphomono-esterase, Enzymologia 15:154, 1951.

2. Albers, H., and Albers, E.: Über die Phosphatasen der Hefe, Ztschr. physiol. Chem. 235:47, 1935.

3. Aldridge, W. N.: Beryllium and Alkaline Phosphatase, Nature, London 165:772, 1950.

4. Amos, H.: The Inactivation of Herpes Simplex by Phosphatase Enzymes, J. Exper. Med. 98:365, 1953.

5. Andrus, M., and Zarrow, M. X.: Amount of Alkaline Phosphatase in the Oviduct of Folic Acid Dificient Chicks, Proc. Exper. Biol. & Med. 72:714, 1949.

6. Atkinson, W. B., and Engle, E. T.: Studies on Endometrial Alkaline Phosphatase During the Menstrual Cycle and in the Hormone Treated Monkey, Endocrinology 40:327, 1947.

7. Atkinson, W. B., and Elftman, H.: Mobilization of Alkaline Phosphatase in the Uterus of the Mouse by Estrogen, Endocrinology 40:30, 1947.

8. Atkinson, W. B.: Effect of Androgen on Alkaline Phosphatase in the Seminal Vesicle of the Mouse, Anat. Rec. 100:731, 1948.

9. Atkinson, W. B., and Gusberg, S. B.: Histochemical Studies on Abnormal Growth of Human Endometrium, Cancer 1:248, 1948.

10. Bailey, K.: Myosin and Adenosinetriphosphatase, Biochem. J. 36:121, 1942.

11. Baker, B. L., and Whitaker, W. L.: Interference with Wound Healing by the Local Action of Adrenocortical Steroids, Endocrinology 40:327, 1947.

12. Barron, E. S. G.: Mechanisms of Carbohydrate Metabolism: An Essay on Comparative Biochemistry, Adv. Enzymol. 3:149, 1943.

13. Bern, H. A.: Effect of Estrogen on Genital Phosphatase Activities in the Male Guinea Pig, Anat. Rec. 108:524, 1950.

14. Bern, H. A.: Estrogen and Alkaline Phosphatase Activity in the Genital Tract of the Male Mouse, Endocrinology 48:25, 1951.

15. Bern, H. A.: Alkaline Phosphatase Activity in Epithelial Metaplasia, Cancer Res. 12:85, 1952.

16. Bern, H. A., and Levy, R. S.: The Effects of Castration, Estrogen Administration, and Methylcholanthrene Carcinogenesis on Phosphatase Activity in the Genital Tract of Male Rats, Guinea Pigs, and Rabbits, Am. J. Anat. 90:131, 1952.

17. Bernhard, A., and Rosenbloom, L.: Ion Exchange Effect on Alkaline Phosphatase of Serum with Reference to Cancer, Science 118:114, 1953.

18. Bielsele, J. J., and Wilson, A. Y.: Alkaline Phosphatase Substrate Specificities in Cultured Normal and Malignant Cells of Mouse, Rat and Fowl, Cancer Res. 11:174, 1951.

19. Biesele, J. J., and Biesele, M. M.: Alkaline Phosphatase in Mouse Skin Under Methycholanthrene Treatment, Cancer Res. 4:751, 1944.

20. Bloch-Frankenthal, L., and Back, A.: Effect of Colchicine on Tumor Growth and Tumor Pyrophosphatase, Proc. Soc. Exper. Biol. & Med. 76:105, 1951.

21. Bodian, D., and Mellors, R. C.: Phosphatase Activity in Chromatolytic Nerve Cells, Proc. Soc. Exper. Biol. & Med. 55:243, 1944.

22. Bourne, G.: The Distribution of Alkaline Phosphatase in Various Tissues, Quart. J. Exper. Physiol. 32:1, 1944.

23. Bourne, G. H.: Alkaline Phosphatase in Taste Buds and Nasal Mucosa, Nature, London 161:445, 1948.

24. Bracet, J.: Nucleic Acids in the Cells and Embryo, Symp. Soc. Exper. Biol. 1:207, 1947.

25. Bradfield, J. R. G.: The Localization of Enzymes in Cells, Biol. Rev. 25:113, 1950.

26. Bradfield, J. R. G.: Alkaline Phosphatase in Invertebrate Sites of Protein Secretion, Nature, London 57:876, 1946.

27. Brues, A. M.; Tracy, M. M., and Cohn, W. E.: Nucleic Acids of Rat Liver and Hepa-

toma: Their Metabolic Turnover in Relation to Growth, J. Biol. Chem. 155:619, 1944.

28. Bunting, H.: Cytochemical Properties of Apocrine Sweat Glands Normally Present in the Human Mammary Gland, Anat. Rec. 101:5, 1948.

29. Bunting, H.; Wislocki, G. B., and Dempsey, E. W.: The Chemical Histology of Human Eccrine and Apocrine Sweat Glands, Anat. Rec. 100:61, 1948.

30. Bunting, H., and White, R. F.: Histochemical Studies of Skin Wounds in Normal and Scorbutic Guinea Pigs, Arch. Path. 49:590, 1950.

31. Cantarow, A., and Miller, L.: Nonexcretion of Jaundice Serum Alkaline Phosphatase in Bile of Normal Dogs, Am. J. Physiol. 153:444, 1948.

32. Charpy, J.: The Treatment of Cutaneous Tuberculosis, Brit. J. Dermat. & Syph. 60:126, 1948.

33. Chase, H. B.: Growth of Hair, Physiol. Rev. 34:113, 1954.

34. Cohn, S. H., and Gong, J. K.: Effect of 2000 Roentgens Local X-Irradiation on Metabolism and Alkaline Phosphatase Activity of Rat Bone, Am. J. Physiol. 173:115, 1953.

35. Danielli, J. F., and Catcheside, D. G.: Phosphatase on Chromosomes, Nature, Lonodn 156:294, 1945.

36. Danielli, J. F.; Fell, H. B., and Kodicek, E.: The Effect of Different Degrees of Vitamin C Deficiency on the Phosphatase in Experimental Wounds in Guinea Pigs, Brit. J. Exper. Path. 26:367, 1945.

37. Danielli, J. F.: A Critical Study of Technique for Determining the Cytological Position of Alkaline Phosphatase, J. Exper. Biol. 22:110, 1946.

38. Danielli, J. F.: Cytologic Demonstration of Alkaline Phosphatase, Nature, London 165:762, 1950.

39. Deane, H. W., and Dempsey, E. W.: The Localization of Phosphatase in the Golgi Region of Intestinal and Other Epithelial Cells, Anat. Rec. 93:401, 1945.

40. Dempsey, E. W., and Wislocki, G. B.: Histochemical Contributions to Physiology, Physiol. Rev. 26:1, 1946.

41. Dounce, A. L.: Enzyme Study on Isolated Cell Nuclei of Rat Liver, J. Biol. Chem. 147:685, 1943.

42. Doyle, W. L.: Effects of Dehydrating Agents on Phosphatases in the Lymphatic Nodules of the Rabbit Appendix, Proc. Soc. Exper. Biol. & Med. 69:43, 1948.

43. DuBois, K. P., and Potter, V. R.: The Assay of Animal Tissues for Respiratory Enzymes: III. Adenosinetriphosphatase, J. Biol. Chem. 150:185, 1943.

44. DuBois, K. P.; Cohran, K. W., and Mazur, M. M.: Inhibition of Phosphatases by Beryllium and Antagonism of the Inhibition by Manganese, Science 110:420, 1949.

45. Elftman, H.: The Effect of Testosterone on the Alkaline Phosphatase of the Adrenal Cortex of the Mouse, Anat. Rec. 97:331, 1947.

46. Elftman, H.: Response of the Alkaline Phosphatase of the Adrenal Cortex of the Mouse to Androgen, Endocrinology 41:85, 1947.

47. Ely, J. O., and Rose, M. H.: Effect of a Protein Free Diet on the Alkaline and Acid Phosphatase Activity of the Liver of the Rat, Nature, London 168:323, 1951.

48. Emmel, V. M.: Alkaline Phosphatase in the Golgi Zone of Absorbing Cells of the Small Intestine, Anat. Rec. 91:39, 1945.

49. Emmel, V. M.: The Intracellular Distribution of Alkaline Phosphatase Activity Following Various Methods of Histologic Fixation, Anat. Rec. 95:159, 1946.

50. Engel, M. B., and Furuta, W.: Histochemical Studies of Phosphatase Distribution in Developing Teeth of Albino Rat, Proc. Soc. Exper. Biol. & Med. 90:5, 1942.

51. Eränkö, O.: Personal communication to the author, 1953.

52. Feigin, I.; Wolf, A., and Kabat, E. A.: Histochemical Studies on Tissue Enzymes: VI. A Difficulty in Histochemical Localization of Alkaline Phosphatase in Nuclei, Am. J. Path. 26:647, 1950.

53. Feigin, I., and Wolf, A.: Nuclear Staining in the Gomori Method for Alkaline Phosphatase, J. Nat. Cancer Inst. 13:250, 1952.

54. Feigin, I., and Wolf, A.: Artifactual Nuclear Staining in the Gomori Method for Demonstrating Alkaline Phosphatase: Differing Mechanism with the Cobalt and Silver Visualization Technics, Lab. Invest. 2:115, 1953.

55. Fell, H. B., and Danielli, H. F.: The Enzymes of Healing Wounds: I. The Distribution of Alkaline Phosphatase in Experimental Wounds and Burns in the Rat, Brit. J. Exper. Path. 24: 196, 1943.

56. Fisher, I., and Glick, D.: Localization of Alkaline Phosphatase in Human Skin Normal and Pathological, Proc. Soc. Exper. Biol. & Med. 66: 14, 1947.

57. Flock, E., and Bollman, J.: Alkaline Phosphatase in the Intestinal Lymph of the Rat, J. Biol. Chem. 175:439, 1948.

58. Flock, E. V., and Bollman, J. L.: The Influence of Bile on the Alkaline Phosphatase Activity of Intestinal Lymph, J. Biol. Chem. 184:523, 1950.

59. Franseen, C. C., and McLean, R.: The Phosphatase Activity of Tissues and Plasma in Tumors of Bone, Am. J. Cancer 24:299, 1955.

60. Freiman, D. G.: Use of Organic Chelating Agent in Histochemical Study of Alkaline Phosphatase Activation, Proc. Soc. Exper. Biol. & Med. 84:338, 1953.

61. French, J. E., and Benditt, E. P.: Observations on the Localization of Alkaline Phosphatase in Healing Wounds, A. M. A. Arch. Path. 57:352, 1954.

62. Maengwyn-Davies, G. D., and Friedenwald, J. S.: Histochemical Studies of Alkaline Phosphatase in Tissues of Rat Using Frozen Section, J. Cell. & Comp. Physiol. 36:421, 1950.

63. Giri, K. V.: Interaction of Vitamin C and Tissue Phosphatases, Biochem. J. 33:309, 1939.

64. Goetsh, J. B.; Reynolds, P. M., and Bunting, H.: Modification of Gomori Method for Alkaline and Acid Phosphatase Avoiding Artefact Staining of Nucleus, Proc. Soc. Exper. Biol. & Med. 80:71, 1952.

65. Gold, N., and Gould, B.: Collagen Fiber Formation and Alkaline Phosphatase, Arch. Biochem. 33:155, 1951.

66. Gomori, G.: Microtechnical Demonstration of Phosphatase in Tissue Sections, Proc. Soc. Exper. Biol. & Med. 42:23, 1939.

67. Gomori, G.: Distribution of Acid Phosphatase in Tissues Under Normal and Under Pathological Conditions, Arch. Path. 32:189, 1941.

68. Gomori, G.: The Distribution of Phosphatase in Normal Organs and Tissues, J. Cell. & Comp. Physiol. 17:71, 1941.

69. Gomori, G.: Hexose Diphosphatase, J. Biol. Chem. 148:139, 1943.

70. Gomori, G.: Calcification and Phosphatase, Am. J. Path. 19:197, 1943.

71. Gomori, G.: Further Studies in the Histochemical Specificity of Phosphatase, Proc. Soc. Exper. Biol. & Med. 72:449, 1949.

72. Gomori, G.: Further Studies on the Histochemical Specificity of Phosphatases, Proc. Soc. Exper. Biol. & Med. 72:440, 1949.

73. Gomori, G.: Histochemical Specificity of Phosphatases, Proc. Soc. Exper. Biol. & Med. 70: 7, 1949.

74. Gomori, G.: Sources of Error in Enzymatic Histochemistry, J. Lab. & Clin. Med. 35:802, 1950.

75. Gomori, G.: Alkaline Phosphatase of Cell Nuclei, J. Lab. & Clin. Med. 37:526, 1951.

76. Gomori, G.: Microscopic Histochemistry: Principles and Practice, Chicago, The University of Chicago Press, 1952.

77. Gomori, G., and Benditt, E. P.: Precipitation of Calcium Phosphate in the Histochemical Method for Phosphatase, J. Histochem. & Cytochem. 1:114, 1953.

78. Gould, B. S., and Gold, N. I.: Studies on the "Alkaline" Phosphatase Associated with Regenerating Connective Tissue Fibers, A. M. A. Arch. Path. 52:413, 1951.

79. Greenstein, J. P.: Recent Progress in Tumor Enzymology, Adv. Enzymol. 3:315, 1943.

80. Grier, R. S.; Hood, M. B., and Hoagland, M. B.: Effects of Beryllium on Alkaline Phosphatase, J. Biol. Chem. 180:289, 1949.

81. Griesemer, R. D., and Gould, E.: A Method for the Study of the Intermediary Carbohydrate Metabolism of Epidermis: I. Oxidation of Acids of the Citric Acid Cycle, J. Invest. Dermat. 22: 299, 1954.

82. Grogg, E., and Pearse, A. G. E.: Coupling Azo Dye Methods for Histochemical Demonstration of Alkaline Phosphatase, Nature, London 170: 578, 1952.

83. Grogg, E., and Pearse, A. G. E.: The Enzymatic and Lipid Histochemistry of Experimental Tuberculosis, J. Exper. Path. 33:567, 1952.

84. Hardy, M. H.: The Histochemistry of Hair Follicles in the Mouse, Am. J. Anat. 90:285, 1952.

85. Herlant, M., and Timeras, P. S.: Alkaline Phosphatase in Various Tissues of the Rat During the Alarm-Reaction, Endocrinology 46:243, 1950.

86. Herman, E., and Deane, H. W.: Comparison of Alkaline Phosphatase Activity in Frozen and Paraffin Sections, J. Nat. Cancer Inst. 13:248, 1952.

87. Hollander, A.; Sommers, S. C., and Grimwade, A. E.: Histochemical and Ultraviolet Microscopic Studies of Chronic Dermatoses and the Corium Membrane, J. Invest. Dermat. 22:335, 1954.

88. Horowitz, N. H.: Histochemical Study of Phosphatase and Glycogen in Fetal Heads, J. Dent. Res. 21:519, 1942.

89. Huggins, C., and Hodges, C. V.: The Effect of Castration, of Estrogen, and of Androgen Injection on Serum Phosphatases in Metastatic Carcinoma of the Prostate, Cancer Res. 1:293, 1941.

90. Jeener, R.: Cytochemical Effects of Oestradial, Nature, London 159:578, 1947.

91. Johansen, G., and Linderstrom-Lang, K.: Liberation, Diffusion, and Precipitation of Phosphate in the Gomori Test, J. Histochem. & Cytochem. 1:442, 1953.

92. Johnson, P. L.; Butcher, E. O., and Bevelander, G.: The Distribution of Alkaline Phosphatase in the Cyclic Growth of the Hair Follicle, Anat. Rec. 93:355, 1945.

93. Johnson, P. L., and Bevelander, G.: Glycogen and Alkaline Phosphatase in the Developing Hoof, Anat. Rec. 99:633, 1947.

94. Johnson, P. L., and Bevelander, G.: The Localization of Alkaline Phosphatase and Glycogen in the Developing Down Feather, Anat. Rec. 98:147, 1947.

95. Kabat, E. A.: Association of Phosphatase with a Material in the Kidney Sedimentable at High Speed, Science 93:43, 1941.

96. Kabat, A., and Furth, J.: A Histochemical Study of the Distribution of Alkaline Phosphatase, Am. J. Path. 17:303, 1941.

97. Kallman, F. G.: Substrate Specificities and Inhibition of Intracellular Alkaline Phosphatases Associated with Cytoplasmic Granules Sedimented by Differential Centrifugation, J. Cell. & Comp. Physiol. 38:137, 1951.

98. Kamell, S. A., and Atkinson, W. B.: Effects of Ovarian Hormones on Certain Cytoplasmic Reactions in the Vaginal Epithelium of the Mouse, Proc. Soc. Exper. Biol. & Med. 68:537, 1948.

99. Kar, A. B.: Studies in the Distribution and Concentration of Alkaline Phosphatase in the Adrenal Cortex of Normal and Sex Hormone Treated Pigeons, Endocrinology 46:363, 1948.

100. Kay, H. D.: Plasma Phosphatase, J. Biol. Chem. 89:249, 1930.

101. Kay, H. D.: Phosphatase in Growth and Disease of Bone, Physiol. Rev. 12:384, 1932.

102. King, E. S.: Plasma Alkaline Phosphatase in Disease, Brit. M. Bull. 9:160, 1953.

103. King, T. S., and Nigrelli, R. F.: Glycerophosphatases of the Normal and Tumerous Frog Kidney, Proc. Soc. Exper. Biol. & Med. 72:373, 1949.

104. Klemperer, F. W.; Miller, J. M., and Hill, C. J.: The Inhibition of Alkaline Phosphatase by Beryllium, J. Biol. Chem. 180:281, 1949.

105. Knox, W. E.: Research in Medical Science, New York, The Macmillan Company, 1950, p. 137.

106. Kochakian, C. D.: Histochemical Study of "Alkaline" Phosphatase of the Kidney of Castrated Mouse After Stimulation with Various Androgens, Am. J. Physiol. 152:257, 1948.

107. Kochakian, C. D., and Fox, R. P.: The Effect of Castration and Testosterone Propionate on the "Alkaline" and "Acid" Phosphatases of Kidney, Liver, and Intestine of the Mouse, J. Biol. Chem. 153:669, 1944.

108. Kochakian, C. D.: The Effect of Various Steroid Hormones on the "Alkaline" and "Acid" Phosphatases of the Kidney of the Mouse, Am. J. Physiol. 145:118, 1945.

109. Krugelis, E. J.: Distribution and Properties of Intracellular Alkaline Phosphatase, Biol. Bull. 90:220, 1946.

110. Kutscher, W., and Wolbergs, H.: Prostataphosphatase, Ztschr. physiol. Chem. 236:237, 1935.

111. Kutscher, W., and Sieg, H.: Über die Wirkgrupper der Phosphatase, Naturwissenschaften 37:451, 1950.

112. Landow, H.; Kabat, E. W., and Newman, W.: Distribution of Alkaline Phosphatase in Normal and in Neoplastic Tissues of the Nervous System, Arch. Neurol. & Psychiat. 48:518, 1942.

113. Lang, W. R.; Rakoff, A. E., and Cross, M.: Alkaline Phosphatase in Vaginal Biopsies, Am. J. Obst. & Gynec. 68:815, 1954.

114. Leduc, E. H., and Dempsey, E. W.: Activation and Diffusion as Factors Influencing Reliability of Histochemical Method for Alkaline Phosphatase, J. Anat. 85:305, 1951.

115. Li, C. H.: The Effect of Hypophysectomy and Adrenocorticotropic Hormone on the Alkaline Phosphatase of Rat Plasma, J. Biol. Chem. 163:715, 1946.

116. Linderbaum, A.; White, M. R., and Shubert, J.: Effect of Aurintricarbolic Acid on Beryllium Inhibition of Alkaline Phosphatase, J. Biol. Chem. 196:273, 1952.

117. Lison, L.: La Recherche histochimique des phosphatases: Étude critique, Bull. hist. appl. et tech. micr. 25:23, 1948.

118. Lora-Tomayo, M., and Alverey, E. F.: Coenzyme of Kidney Phosphatase, Nature, London 173:548, 1954.

119. Lorch, J.: Alkaline Phosphatase and the Mechanism of Ossification, J. Bone & Joint Surg. 31B:94, 1949.

120. Ludwig, S., and Charnutin, A.: Effect of X-Ray Irradiation on Alkaline Phosphatase of Plasma and Tissues of Rats, Am. J. Physiol. 163: 648, 1950.

121. Ludwig, A. W., and Boas, N. F.: The Effect of Testosterone on the Connective Tissue of the Comb of the Cockerel, Endocrinology 46:29, 1950.

122. Madsen, N. B., and Tuba, J.: The Role of Alkaline Phosphatase in Intestinal Absorption: I. The Kinetics of Phosphatase Action on Various Substrates, Canad. J. M. Sc. 31:1, 1953.

123. Manheimer, L. H., and Seligman, A. M.: Improvement in the Method for the Histochemical Demonstration of Alkaline Phosphatase and Its Use in a Study of Normal and Neoplastic Tissue, J. Nat. Cancer Inst. 9:181, 1948.

124. Martin, B. F., and Jacoby, F.: Diffusion Phenomenon Complicating the Histochemical Reaction for Alkaline Phosphatase, J. Anat. 83:351, 1949.

125. Menten, M. J.; Junge, J., and Green, M. H.: A Coupling Histochemical Azo Dye Test for Alkaline Phosphatase in the Kidney, J. Biol. Chem. 153:471, 1944.

126. Mescon, H.; Gray, M., and Moreth, G.: Molluscum Contagiosum: A Histochemical Study, J. Invest. Dermat. 23:293, 1954.

127. Molnár, J.: The Use of Rhodizonate in Enzymatic Histochemistry, Stain Technol. 27:221, 1952.

128. Monacelli, M., and Ribuffo, A.: Der Hautzuckergehalt bei der Psoriasis, Hautarzt 3:498, 1952.

129. Montagna, W., and Noback, C. R.: The Histochemistry of the Preputial Gland of the Rat, Anat. Rec. 96:111, 1946.

130. Montagna, W.: Histochemical Analysis of the Sebaceous Glands of the Hamster, Anat. Rec. 99:560, 1947.

131. Montagna, W.: Histochemistry of the Sebaceous Glands of the Rat, Anat. Rec. 97:356, 1947.

132. Montagna, W., and Hamilton, J. B.: Histochemical Analysis of the Sebaceous Glands of the Hamster, Anat. Rec. 99:560, 1947.

133. Montagna, W., and Hamilton, J. B.: Histochemical Analysis of the Sebaceous Glands of the Hamster, Fed. Proc. 6:166, 1947.

134. Montagna, W., and Noback, C. R.: Histochemical Observations on the Sebaceous Glands of the Rat, Am. J. Anat. 81:39, 1947.

135. Montagna, W.; Noback, C. R., and Zak, F. G.: Pigment, Lipids, and Other Substances in the Glands of the External Auditory Meatus of Man, Am. J. Anat. 83:409, 1948.

136. Montagna, W., and Parks, H. F.: A Histochemical Study of the Glands of the Anal Sac of the Dog, Anat. Rec. 100:297, 1948.

137. Moog, F.: Localizations of Alkaline and Acid Phosphatases in the Early Embryogenesis of the Chick, Biol. Bull. 86:51, 1944.

138. Moog, F.: The Physiological Significance of the Phosphomonoesterases, Biol. Rev. 21:41, 1946.

139. Moog, F., and Wenger, E. L.: The Occurrence of a Neutral Mucopolysaccharide at Sites of High Alkaline Phosphatase Activity, Am. J. Anat. 90:339, 1952.

140. Morse, A., and Green, R. O.: Alkaline Glycerophosphatase in the Developing Teeth of the Rat, Anat. Rec. 99:379, 1947.

141. McCollum, E. V., and Hart, E. B.: On the Occurrence of a Phytin-Splitting Enzyme in Animal Tissues, J. Biol. Chem. 4:497, 1908.

142. McKelvin, A. M., and Mann, F. C.: The Role of Alkaline Phosphatase in Osteogenesis After Transplantation of Bone, Proc. Staff. Meet. Mayo Clin. 23:449, 1948.

143. McManus, J. F.; Lupton, W. M., and Harden, G.: Histochemical Studies of 5 Nucleotidase, Lab. Invest. 1:488, 1952.

144. Neumann, H.: Dependence of the pH Optimum of the Phosphomonoesterase on the Substrate Concentration and on Inhibitors and Activators, Biochim. et biophys. acta 3:117, 1949.

145. Newman, W.; Feigin, I.; Wolf, A., and Kabat, E.: Histochemical Studies of Tissue Enzymes: IV. Distribution of Some Enzyme Systems Which Liberate Phosphate at pH 9.2 as Determined with Various Substrates, and Inhibitors; Demonstration of 3 Groups of Enzymes, Am. J. Path. 26:257, 1950.

146. Novikoff, A. B.: Validity of Histochemical Phosphatase Methods on Intracellular Level, Science 113:320, 1951.

147. Oppenheimer, M., and Flock, E.: Alkaline Phosphatase Levels in Plasma Following Partial Hepatectomy, Am. J. Physiol. 149:418, 1947.

148. Paff, G. H., and Montagna, W.: Cytochemical Studies of Normal and Tumor Mast Cells, Cancer Res. 7:798, 1947.

149. Pearse, A. G. E.: Modern Methods in Histochemistry, J. Clin. Path. 4:1, 1951.

150. Pearse, A. G. E., and Ries, J. L.: Histochemical Demonstration of Specific Phosphatase, Biochem. J. 50:534, 1952.

151. Pearse, A. G. E., and Ries, J. L.: The Histochemical Demonstration of a Specific Phosphatase (5 Nucleotidase), Biochem. J. 50:534, 1952.

152. Pearse, A. G. E.: Histochemistry Theoretical and Applied, Boston, Little, Brown & Company, 1953.

153. Pearson, B.; Novikoff, A. D., and Morrione, T. G.: Histochemical Localization of Alkaline Phosphatase During Carcinogenesis in Rats fed P-Demethyl Amino Azobenzene, Cancer Res. 10:557, 1950.

154. Pearson, B., and Richardson, F.: Difference in Alkaline Phosphatase Activity and Distribution Among Several Transplantable Mammary Tumors in Mice, Cancer Res. 12:287, 1952.

155. Perry, S. V.: Adenosintriphosphatase Activity of Myofibrils Isolated from Skeletal Muscle, Biochem. J. 48:257, 1951.

156. Pirilä, V., and Eränkö, O.: Distribution of Histochemically Demonstrable Alkaline Phosphatase in Normal and Pathologic Human Skin, Acta path. et microbiol. scandinav. 27:650, 1950.

157. Ries, J. L.: La Nucléotidase et sa relation avec la désamination des nucléotides dans le coeur et dans le muscle, Bull. Soc. chim. biol. 16:385, 1934.

158. Ries, J. L.: Specificity of Phosphomonesterases in Human Tissues, Biochem. J. 48:548, 1951.

159. Ring, J. R.: Alkaline Phosphatase Activity of Oral and Vaginal Epithelium of the Rat During the Estrous Cycle, Anat. Rec. 103:498, 1949.

160. Ring, J. R., and Levy, B. M.: Changes in Alkaline Phosphatase Activity of Rat Oral Epithelium During Estrous Cycle and in Response to Administered Estrogen, J. Dent. Res. 29:817, 1950.

161. Robertson, W. V.; Dunihue, F. W., and Novikoff, A. B.: Metabolism of Connective Tissue, Absence of Alkaline Phosphatase in Collagen Fibers During Formation, Brit. J. Exper. Path. 31:545, 1950.

162. Robertson, W. B.; Dunihue, F. W., and Novikoff, A. G.: The Metabolism of Connective Tissue: Absence of Alkaline Phosphatase in Colla-

gen Fibers During Formation, Brit. J. Exper. Path. 31:545, 1950.

163. Roche, J. Blood Phosphatases, Biochem. J. 25:1724, 1931.

164. Roche, J.: Le Rôle des phosphatases dans le métabolisme du calcium, Ann. nutrit. alimentation 1:3, 1947.

165. Roche, J.: The Enzymes, edited by J. B. Sumner and K. Myrback, New York, Academic Press, Inc., 1950, Vol. 1, Pt. 1, p. 473.

166. Rosenthal, O.; Fahl, J., and Wells, E.: Changes in Concentration and Type of Hepatic Alkaline Phosphatase Due to Dietary Deficiency and Regeneration, Fed. Proc. 10:239, 1951.

167. Ross, M. H.: Alkaline Phosphatase Activity and pH Optima, J. Biol. Chem. 192:561, 1951.

168. Rothman, S.: Physiology and Biochemistry of the Skin, Chicago, University of Chicago Press, 1954.

169. Ruyter, J. H. C., and Neumann, H.: A Critical Examination of the Histochemical Demonstration of the Alkaline Phosphomonoesterase, Biochim. et Biophys. Acta 3:125, 1949.

170. Sadasivan, V.: Nature of Phosphatase Activity, Nature, London 169:418, 1952.

171. Sadasivan, V.: Zinc, Ionic Equilibrium and Phosphatase Activity, Nature, London 170:421, 1952.

172. Seligman, A. M., and Manheimer, L. H.: A New Method for the Histochemical Demonstration of Acid Phosphatase, J. Nat. Cancer Inst. 9:427, 1948.

173. Shelly, W. B., and Mescon, H.: Histochemical Demonstration of Secretory Activity in Human Eccrine Sweat Glands, J. Invest. Dermat. 18:289, 1952.

174. Stafford, R. O., and Atkinson, W. B.: Effect of Acetone and Alcohol Fixation and Paraffin Embedding on Activity of Acid and Alkaline Phosphatases in Rat Tissue, Science 107:279, 1948.

175. Stein, S., and Koshland, D.: Mechanism and Action of Alkaline Phosphatase, Arch. Biochim. & Biophys. 39:229, 1952.

176. Sumner, J. B., and Somers, G. F.: Chemistry and Methods of Enzymes, Ed. 7, New York, Academic Press, Inc., 1947.

177. Sumner, J. B., and Myrback, K.: The Enzymes, New York, Academic Press, Inc., 1950, Vol. 1, Pt. 1.

178. Suskind, R.: The Chemistry of the Human Sebaceous Gland, J. Invest. Dermat. 17:37, 1951.

179. Suzuki, U.; Yoshimura, K., and Takaishi, M., cited by Kay, H. D.: Phosphatase in Growth and Disease of Bone, Physiol. Rev. 12:384, 1932.

180. Swanson, M. A.: Phosphatases of Liver: I. Glucose-6-Phosphatase, J. Biol. Chem. 184:647, 1950.

181. Swanson, M.: Alkaline Phosphatases of Liver, So-Called Adenosintriphosphatases, J. Biol. Chem. 191:577, 1951.

182. Szirmai, J. A.: The Effect of Testosterone Propionate on the Connective Tissue of the Head, Appendices and Skin of the Capon, Anat. Rec. 105:337, 1949.

183. Takamatsu, H.: Manshu Igaku Zasshi 31: 34, 1938; cited by Gomori, G.: Microscopic Histochemistry: Principles and Practice, Chicago, University of Chicago Press, 1952, p. 180.

184. Takamatsu, H.: Histologische und biochemische Studien über die Phosphatase: Histochemische Untersuchungsmethodik der Phosphatase und deren Verteilung in verschiedenen Organen and Geweben, Tr. Soc. path. japon. 29:492, 1939.

185. Talmadge, R. V.: Influence of Estradiol on Alkaline Phosphatase Activity in the Genital Tract of the Rat, Proc. Soc. Biol. & Med. 70:719, 1949.

186 Tamayo, M., and Municlo, A.: Coenzyme of Kidney Phosphatase, Nature, London 168:249, 1951.

187. Valentine, W. N.; Follette, J. H.; Harolin, E. B.; Beck, W. S., and Lawrence, J. S.: Studies on Leucocyte Alkaline Phosphatase Activity: Relation to "Stress" and Pituitary-Adrenal Activity, J. Lab. & Clin. Med. 44:219, 1954.

188. Veerkamp, R. A., and Smits, G.: Mode of Action of Beryllium on Alkaline Phosphatase, Nature, London 172:589, 1953.

189. Washstein, M., and Meisel, E.: The Histochemical Distribution of 5-Nucleotidase and Unspecific Alkaline Phosphatase in the Testicle of Various Species and Human Seminomas, J. Histochem. & Cytochem. 2:137, 1954.

190. Wang, K. S., and Grossman, M. I.: Histochemical Study of Alkaline Phosphatase in Tissue Prepared by the Freezing-Drying Technique, Anat. Rec. 104:79, 1949.

191. Washburn, W. W.: Comparative Histochemical Observations on Wound Healing in Adult Rats and Cultured Adult Human Epithelium, J. Invest. Dermat. 23:97, 1954.

192. Yokoyama, H. O.; Stowell, R., and Mathews, R.: Evaluation of Histochemical Alkaline Phosphatase Techniques, Anat. Rec. 109:139, 1951.

193. Zetterström, R.: Phosphorylation of Vitamin D2: Action of Phosphorylated Compound in Alkaline Kidney Phosphatase, Nature, London 167:409, 1951.

194. Zorzoli, A., and Nadel, E. M.: A Histochemical and Biochemical Study of the Alkaline Phosphatase Activity of Bone of Scorbutic Guinea Pigs, J. Nat. Cancer Inst. 10:1366, 1950.

195. Zorzoli, A., and Nadel, E. M.: Alkaline Phosphatase Activity in the Bones of Normal and Scorbutic Guinea Pigs, J. Histochem. & Cytochem. 1:362, 1953.

Illustrations

The following abbreviations are used in the legends: (1) H & E, hematoxylin and eosin stain; (2) AP, alkaline phosphatase specimen incubated with glycerophosphate substrate; (3) AP-C, alkaline phosphatase specimen incubated with glycerophosphate substrate counterstained with lithium carmine.

Fig. 1.—Normal papillary capillaries. AP stain; reduced from mag. × 428. Moderate phosphatase activity of all capillaries. Note: Branching afferent capillary (*1*), complete capillary loop (2), and efferent collecting vessel (*3*). Some melanin pigment is seen in the basal layer of the epidermis, but no AP activity is seen in the epidermis (*4*) or connective tissue (*5*).

Fig. 2.—Elongated capillary loop. AP-C stain; reduced from mag. × 428. Specimen of a prickle-cell epithelioma with acanthotic epidermis. A keratin "pearl" with false positive deposit is seen to the lower left. Some phosphatase activity (mainly nuclear) is seen in the inflammatory cells neighboring the capillary.

Fig. 3.—Psoriasis. AP-C stain; reduced from mag. × 150. False positive precipitate, principally nuclear, of parakeratotic horn (*1*). Substantial AP activity of exocytotic cells forming an exaggerated Monro's microabscess (*2*). AP-active papillary capillaries (*3*).

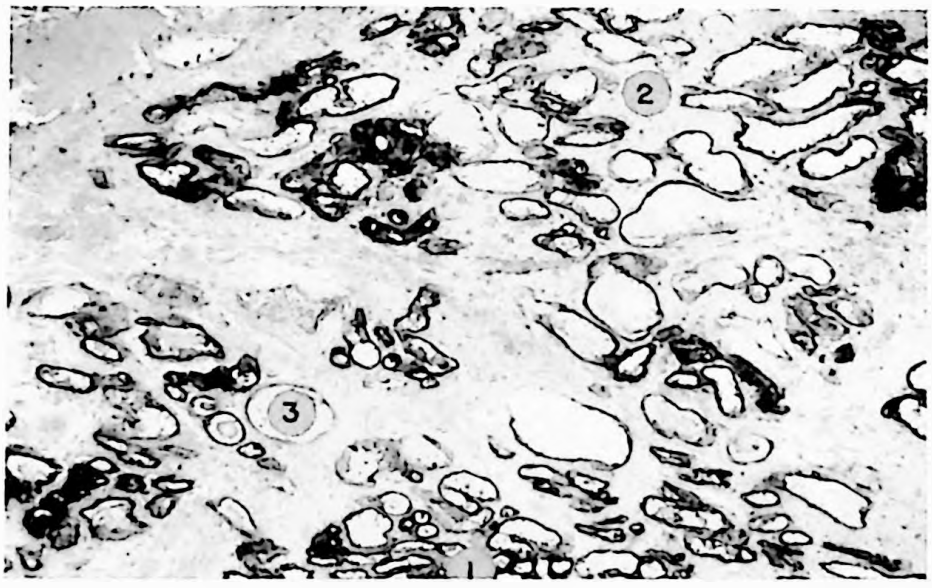

Fig. 4.—Hemangioma. AP-C stain; reduced from mag. × 150. Smallest vessels (*1*) show moderate AP activity, whereas medium-sized vessels (*2*) have less, and largest (dilated) vessels (*3*) show least. The stroma is negative.

Fig. 5. — Granuloma pyogenicum. AP stain; reduced from mag. × 41. Moderate AP activity is seen in relation to the numerous vessels and to the proliferating fibrous tissue cells of the stroma.

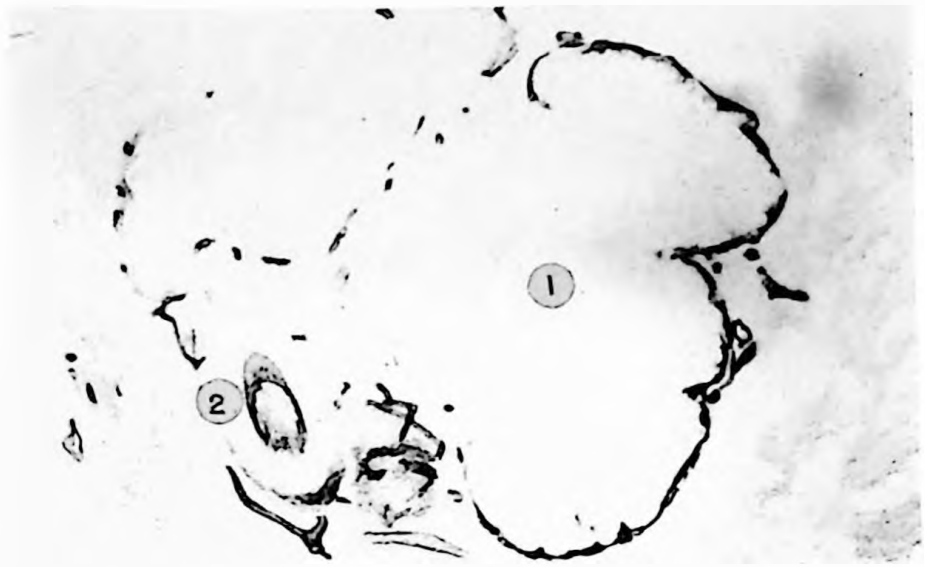

Fig. 6. — Sebaceous gland with its surrounding vascular network. AP stain; × 150. The sebaceous gland (*1*) itself shows no AP activity and therefore is defined only by the AP-positive delicate network surrounding it. Cross section of a hair follicle is seen at (*2*), with false positive deposit in the inner root sheath.

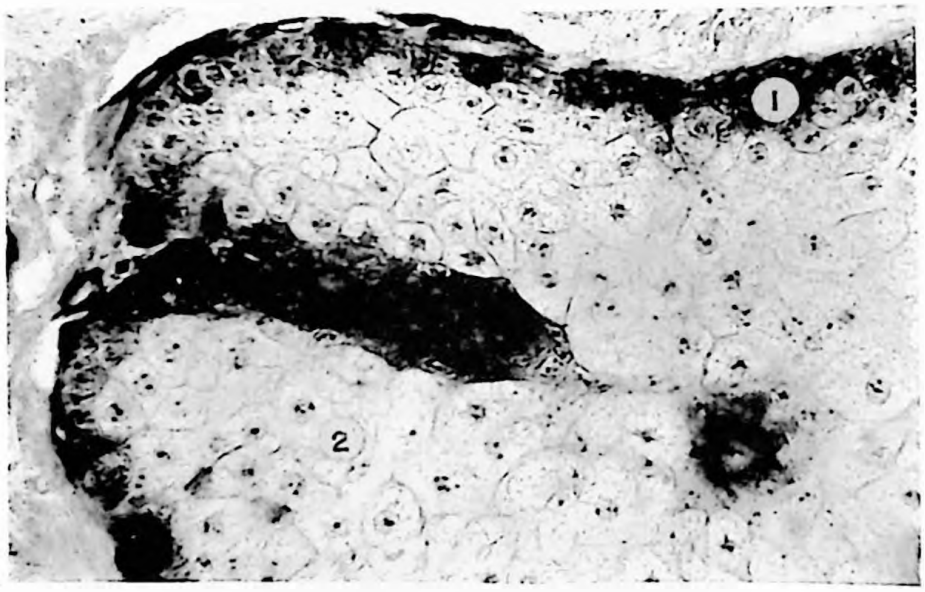

Fig. 7.—Sebaceous adenoma. AP-C stain; reduced from mag. × 428. The peripheral acinar cells of the sebaceous lobule (*1*) show moderate AP activity, both cytoplasmic and nuclear. The acinar cells towards the center of the lobule (*2*) have faint (mostly nucleolar) activity.

Fig. 8.—Normal hair follicular apparatus. AP-C stain; reduced from mag. × 185. The hair papilla and external connective tissue sheath show intense AP activity (*1*). The hair bulb matrix cells have moderate activity (*2*). The inner root sheath shows intense false positive cobalt sulfide in both Henele's and Huxley's layers (*3*). The hair shaft and external root sheath are AP-negative.

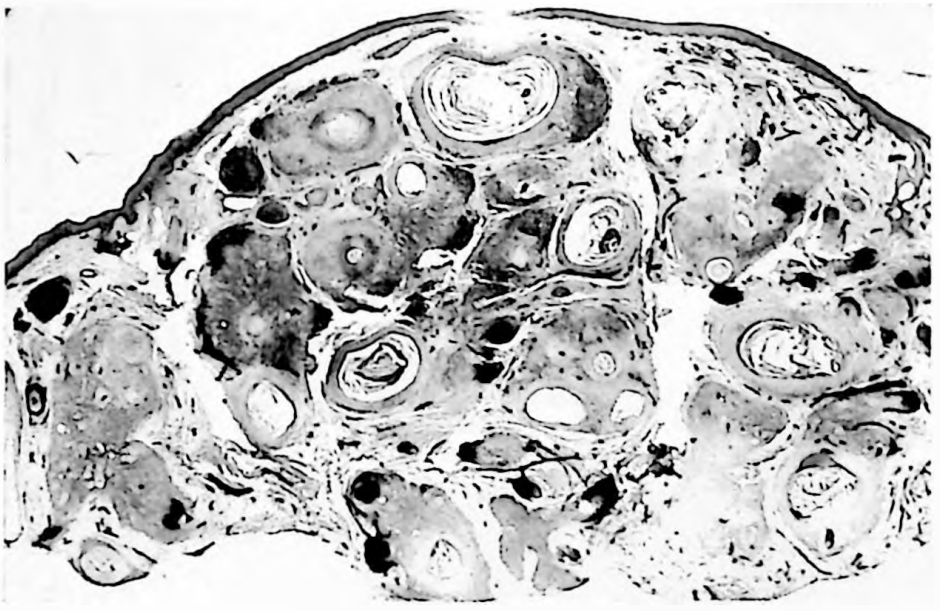

Fig. 9. — Trichoepithelioma. AP-C stain; reduced from mag. × 43. At the periphery of most of the "basal"-cell masses numerous highly AP-active hair papillae are seen. Note that these distorted follicular structures are not continuous with surface epidermis, but are distributed in a haphazard manner. Some horn-cyst-follicular elements show papillae at more than one focal area of the periphery.

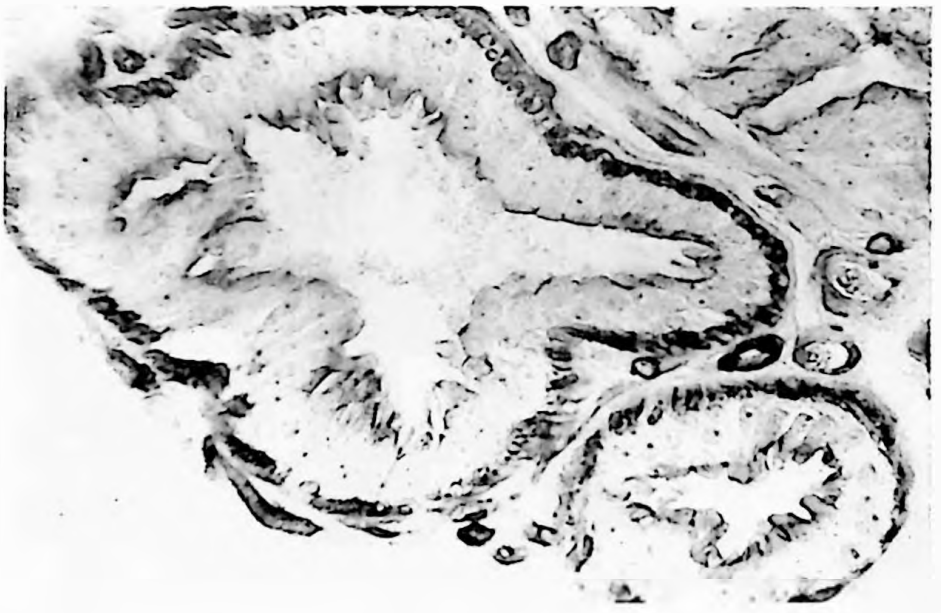

Fig. 10. — Apocrine gland. AP stain; reduced from mag. × 498. The myoepithelial cells surrounding the acinar portion are moderately active. The secretory cells have less intense, bipolar AP activity and some nucleolar activity. The contents of the lumens show mild phosphatase activity. Active capillaries surround the gland.

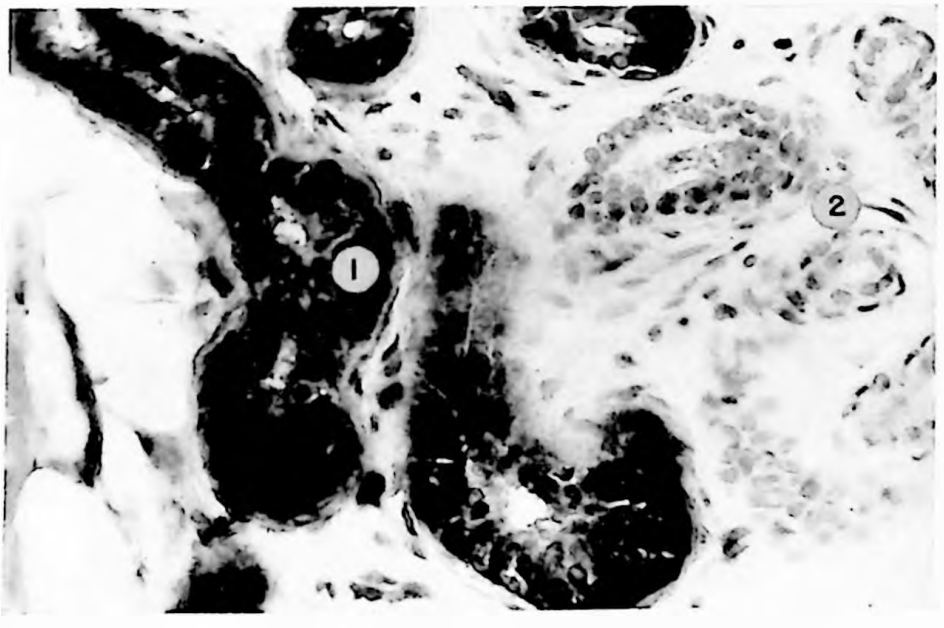

Fig. 11.—Eccrine gland and duct. AP-C stain; reduced from mag. × 428. The myoepithelial cells as well as the secretory cells are intensely AP-active (*1*). The sweat ducts (*2*) are devoid of AP activity (their nuclei are stained with lithium carmine).

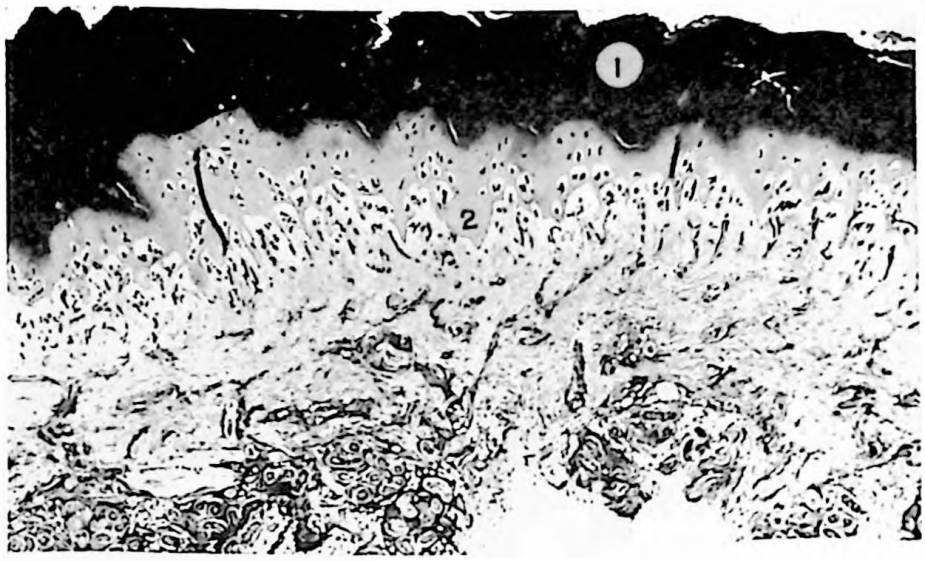

Fig. 12.—Normal sole. AP-C stain; reduced from mag. × 29. Heavy false positive deposit in stratum corneum (*1*). Numerous active papillary capillaries and deeper vessels (*2*). Many AP-active eccrine sweat glands are seen in the subcutis, while the sweat ducts have no AP activity.

Fig. 13.—Hydrocystoma. AP-C stain; reduced from mag. × 428. The cells lining the cyst show substantial AP activity, particularly in the layer lining the lumen. Cobalt sulfide deposit is seen in the contents of the cyst (see text).

Fig. 14.—Erythema induratum. AP stain; reduced from mag. × 23. The central necrotic area is almost devoid of activity (*1*). Peripherally there is intense AP activity in the inflammatory cells, vessels, and connective tissue fibers (*2*). The endothelium of some of the deeper thickened vessels is active.

Fig. 15.—Intradermal nevus. AP-C stain; reduced from mag. ×41. Intense AP activity is seen in relation to certain of the nevus cell groups, while others (those towards the right) have no activity.

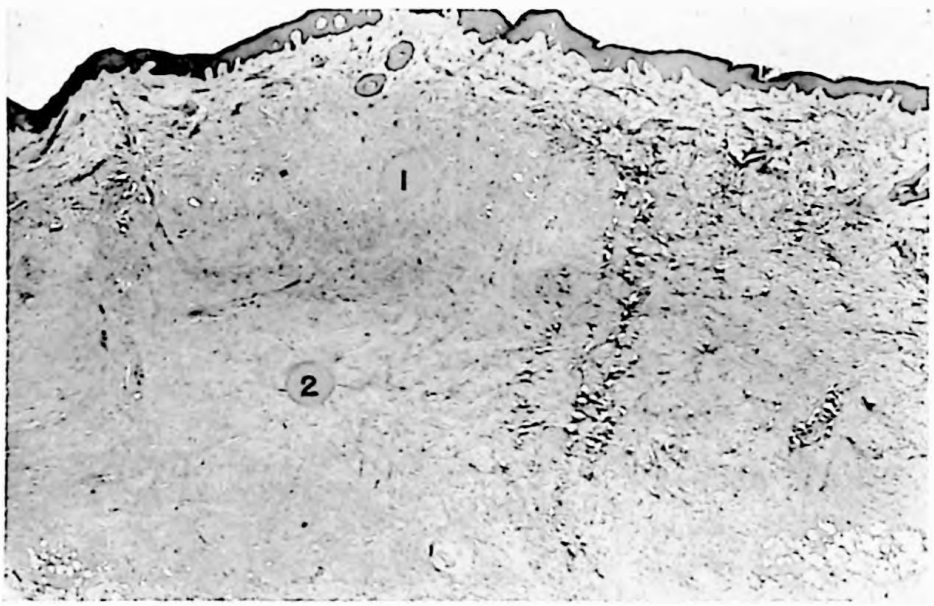

Fig. 16.—Cutaneous nodule. H & E stain; reduced from mag. ×29. The tumor elements of the upper cutis (*1*) are highly cellular and are composed of short spindle, triangular, or irregular cells (Type I cells). The lower cutis (*2*) shows numerous long spindle-shaped cells (Type II cells).

Fig. 17.—Cutaneous nodule. AP stain; reduced from mag. ×29. Same specimen as Figure 16. In the upper cutis the area composed of Type I cells (*1*) shows AP activity only in relation to the capillaries. The deeper cutis (*2*) shows widespread AP activity, not only in relation to the vessels but also to the long spindle cells (Type II cells).

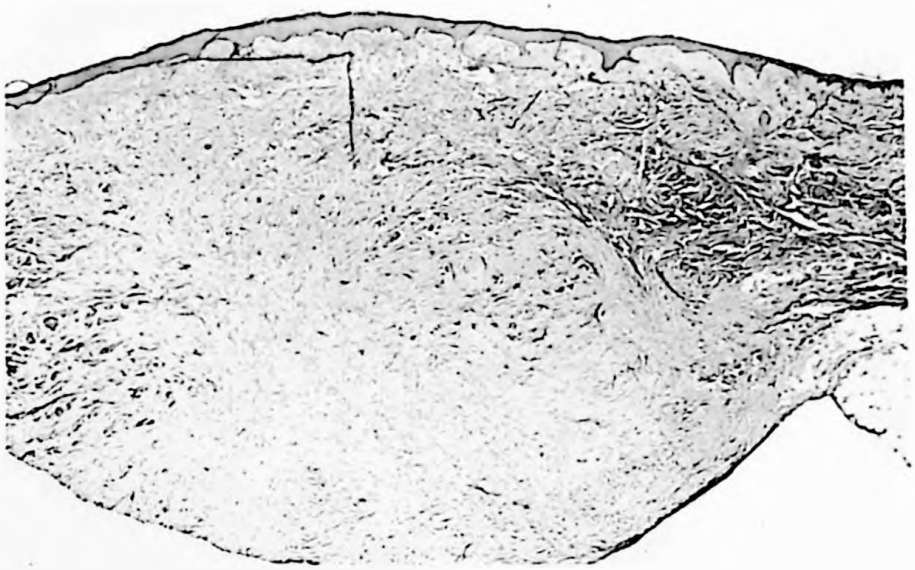

Fig. 18A.—Cutaneous nodule. H & E stain; reduced from mag. × 22. The cutaneous nodule is composed only of long spindle-shaped fibroblasts (Type II cells).

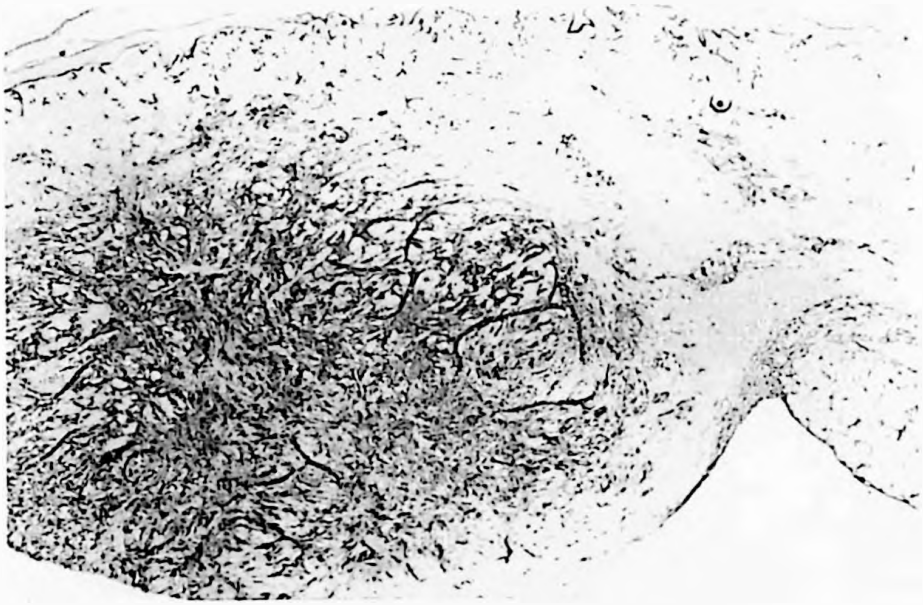

Fig. 18B.—Cutaneous nodule. AP stain; reduced from mag. × 22. This lesion (same specimen as Fig. 18A) is composed entirely of Type II cells (long spindle cells) which show AP activity almost as intensely as that seen in the capillaries.

Fig. 19.—Multiple idiopathic hemorrhagic sarcoma of Kaposi. AP stain; reduced from mag. × 56. This is in the sarcomatous stage. The upper cutis shows numerous highly active capillaries in the loose granulation tissue. The deeper cutis has intense AP activity in relation to the proliferating spindle-cell sarcomatous tissue.